STEPHANIE ELIZABETH MOHR

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Drosophila Research and Biological Discovery

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For my family

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PREFACE

MY FIRST JOB IN THE SCIENCES WAS A WORK-STUDY POSITION AT Wesleyan University. I was a freshman at the time, and the way I remember it now, there were only a few job options available. Most of my dormmates went for positions at the library reshelving books. But I took a position as dishwasher for the department of biology.

It was the perfect job. I would arrive in the afternoon and the dishes would be waiting, prerinsed and sitting in big white plastic tubs, each tub labeled in bold black Sharpie marker with the last name of the professor who led the lab. I was asked to run the dishes from one lab separately from the next, filling the machine with the dishes from one tub at a time. Once I'd loaded the dishes into the industrial-grade washer, all I had to do was throw in a scoop of soap powder and get things going. After that, I could sit and study. Roughly an hour after I had pushed the start button, a buzzer would go off, at which point I would pull on a pair of heat-resistant gloves, unload that set of dishes, and set up the next run.

By the end of my freshman year I was being encouraged to join a lab as an undergraduate researcher. A biochemist whose lab was next door to the small room that held the dishwasher and a couple of autoclaves—high-pressure, high-temperature sterilization machines—would occasionally pop his head in to ask me about joining a lab. He seemed determined

to make sure I knew that I did not have to be sitting around all afternoon doing dishes. Instead, I could be doing hands-on research, either for course credit or for the same kind of work-study pay I was collecting for the dish-washing job. Eventually, and somewhat reluctantly—being paid mostly to do homework was a pretty plum job after all—I decided to take his advice and approach one of the professors in the biology department about a research position.

Starting out, I did not have a clear idea about what kind of research I wanted to do or whose lab I wanted to join. But I did have one firm "no" in my mind. I had decided, quite resolutely, that I would not work for the one lab in the department in which the researchers performed their studies with the fruit fly, *Drosophila melanogaster.*

Why was I so determined that I would not work in a fruit fly lab?

It was a decision based solely on the dishes.

After washing the dishes week after week for the dozen or so labs in the department, I had noticed a pattern. The dishes from most labs were varied: tall glass cylinders, low flasks, wide-mouthed beakers. Now and then a metal spatula or a magnetic stir bar. But there was one exception. Michael Weir was the only professor in the department at that time whose lab worked on fruit flies, and instead of containing the usual odd mix of sizes and shapes, the white trays marked "Weir," I noticed, contained rows and rows of same-sized, same-shaped, half pint glass bottles, many of which were marked "urine specimen" in raised letters.

It wasn't a gross-out factor for me—I knew that there was no urine involved, and the dishes arrived precleaned. No signs of flies or fly larvae. Not even any traces of fly food. Just the rinsed bottles, waiting for a deep mechanical scrub. So why did I object? For one simple reason: the dishes from that lab were too boring. Half pint bottle after half pint bottle loaded into the machine and then, sparkling clean, loaded back into the white plastic tray. I wanted to work for an exciting lab. A lab with a lot going on. A lab that did a lot of different things. I had a unique vantage point from which to judge. And bottle after bottle, all the same size, just didn't cut it.

The decision not to join a fly lab as an undergraduate seems now like the delay of something inevitable, the way two people who have just met and fallen in love might say, "And we lived just around the corner from each other for years!" Even in graduate school at the University of Colorado at Boulder, I circled around for a while before landing. I spent a couple of months working with *Chlamydomonas reinhardtii,* a one-celled green

alga that can breaststroke its way through a liquid with its pair of arm-like cilia, and another couple of months working with *Caenorhabditis elegans,* the small clear nematode worm that, like *Drosophila,* has become a common "model organism"—that is, a species many researchers use in their studies with the assumption (the justifiable assumption, I will argue) that what is learned using the model can be more widely applied to understanding the principles, mechanisms, and genetic underpinnings that control the development, growth, life, health, aging, behavior, and so on of other living creatures, including ourselves.

I was making a kind of Hail Mary throw when I approached Professor Robert Boswell in the early summer of 1994. I was nearing the end of my first year in graduate school—the only year in which classes, rather than lab work, were the main focus. I had been turned down at one lab, and I wasn't keen to join any of the others in which I had done rotations. I was nearly ready to give up on graduate school altogether and head back to the East Coast. I had a job lined up and everything.

But of course the reason quarterbacks still throw Hail Mary passes is that once in a while they pay off, and pay off big. The right things connect and the team makes that dream play. With the decision to give it one last try in Professor Boswell's lab—a *Drosophila* lab—I found the right fit for my graduate thesis and, as it turned out, far beyond. With that decision, I became a "fly person," as those of us who use *Drosophila melanogaster* in our research really do call ourselves.

As model organisms go, the fruit fly is, in my view—and I am not the first to propose this—the model of models. Left to its own devices, this innocuous little fly would be content to spend life doing nothing much more than hovering around a pile of rotting apples or wandering a vineyard. But research on this fly, which began more than a hundred years ago, has helped to reveal a surprising number of fundamental concepts related to biology, including fundamentals of human biology and disease. Although many years have passed since I was first introduced to the field, I continue to be amazed by what research on fruit flies reveals to us. I have gained a deep appreciation for this tiny sprite and for the many researchers who have used *Drosophila* to conduct studies in fields like genetics, genomics, cell biology, developmental biology, and neuroscience.

With this book, I hope to share some of that amazement and appreciation by telling some of the "first in fly" stories—that is, to present some of the key biological insights and discoveries for which *Drosophila* played a starring

role, starting researchers in other fields down a revealing new experimental path they might otherwise not have taken or not stumbled upon so quickly. My goal is not to present a comprehensive list of discoveries made first in the fly or to rigidly adhere to presenting only this type of discovery. Instead, my goal is to use "firsts" as jumping-off points to demonstrate some of the ways in which fly research has broadly impacted our understanding of biology and biomedicine, including our understanding of the functions of human genes. Indeed, for a surprisingly large number of human genes, the first hints of their biological functions—the cellular mechanisms of their action, the answer to "How does it work?"—came not from studies in human cells, mice, rats, or guinea pigs, but instead from studies performed with the lowly fruit fly.

At the 41st Annual *Drosophila* Research Conference in 2000, the opening session was centered around discussion of the newly released full-genome sequence for *Drosophila melanogaster,* the first full-genome sequence of any critter with eyes and legs. During that opening session of the conference, a leader in the fly field joked in front of the crowded auditorium that flies are basically "little people with wings."

They are not, of course.

But they are marvels. And they are connected to us. If by nothing else, then by a thread of evolution, by the fact that Nature tends to solve a problem once and then apply that solution over and over, rather than reinvent the wheel with each new species.

The reasons researchers came to study *Drosophila melanogaster* are to some extent a product of random chance and circumstance. But the reasons that research on fruit flies continues are anything but random. Once exposed to some of the "first in fly" stories—many of them closely connected to the story of our bodies, our behaviors, and our many plagues and diseases—one cannot help seeing that *Drosophila* is more than worthy of the attention it has been given and continues to receive.

FIRST IN FLY

INTRODUCTION

TO LOOK AT A VIAL OF FRUIT FLIES, IN VARIOUS STAGES OF DEVELOPMENT and cultured on typical laboratory fly food—corn meal, yeast, sugar, water, and a bit of agar to hold it together—is to see a lot of neutral and forgettable colors. The food is beige. The minute eggs and small larvae are white. As the larvae grow, they crawl up the sides of the vials, which are stoppered with white fluffs of cotton, and form pupae, which are off-white at first, then darken to shades of amber or buff. When an adult fly is about to emerge, two charcoal gray smudges become visible under the translucent pupal case. These are the wings, wet and folded, ready to expand and dry clear when the fly crawls out into the world. The one thing that makes a fruit fly stand out from this sea of neutral color—white, beige, and charcoal—is the pair of eyes on an adult fly, which, on a "wildtype" or normal fly, are dark red. A pair of garnet jewels, faceted like a geodesic dome (though that is not visible without a microscope), and intense.

A researcher could sort through thousands, tens of thousands, hundreds of thousands of fruit flies and each time see a fly with these dark red eyes and other features in common with its siblings, such as the stereotyped pattern of bristles (small hairs) on its body, the color of its hard external cuticle, or the shape of its wings. But every once in a while the researcher might stumble upon a fly that is notably different, with one or more visible

features that distinguish it from its siblings. One of the first mutations in the fruit fly to be isolated in the lab, bred, and reported in the scientific literature was a mutation that causes the normally dark red eyes to be colorless, or white (Sturtevant 1965). The fly was found in the laboratory of T. H. Morgan, who would go on to win a Nobel Prize. Though he kept flies in the lab, Morgan was not an entomologist interested in insects; he was an embryologist, interested in development and heredity. Morgan had just begun investigating whether flies could be useful to his research when the white-eyed fly started him down the path that would define his career. The fly was bred to a mate. Would the offspring, too, have white eyes? The answer was yes. It must have been a thrill to see that something different—that odd trait—reappear in a next generation of flies.

The gene that was altered in the white-eyed strain of flies was later named *white,* part of an historic system for naming *Drosophila* genes that has for the most part endured, and has a backward kind of logic—the equivalent of naming a car's engine *won't go* or the kitchen faucet *no water.* When the *white* gene functions normally, the fly makes the two types of pigments that together result in the particular shade of red of a wildtype fly eye: bright red pigments called drosopterins and brown pigments called ommochromes. Without the normal function of the *white* gene, neither the bright red nor the brown pigments get made.

As it turns out, the white-eyed flies, first reported in the literature in 1910 (Morgan 1910), were just the beginning.

Morgan subsequently made findings using the *white* mutant flies that were informative—disruptive even—to science's then incipient understanding of genetics, and served to encourage others to use fruit flies in genetic research. The fruit fly *Drosophila melanogaster* eventually became a favorite research subject, with tens, hundreds, and eventually thousands of researchers capitalizing on its many advantages for genetic research. As a result of more than a century of studies, and although countless mysteries remain, *Drosophila* has become what can easily be argued is the best-understood multicellular creature on the planet: we have a more complete picture of the extents of its genes along chromosomes, the functions of those genes, how those genes control development (that is, growth from a fertilized egg to a fully formed adult), the range of behaviors and cellular responses the fully developed fly can display, and so on regarding this one species than we have for any other living creature on the planet. Many students are first introduced to genetic research through *Drosophila* lab studies performed in high school or under-

graduate biology classes, and the term "Drosophila" or "fruit fly" is found in the indexes of most introductory college textbooks on biology. Results from research performed using the fruit fly are routinely featured in high-profile scientific journals, and on occasion make it into the pages of mass media publications like the *New York Times* and *Wall Street Journal*.

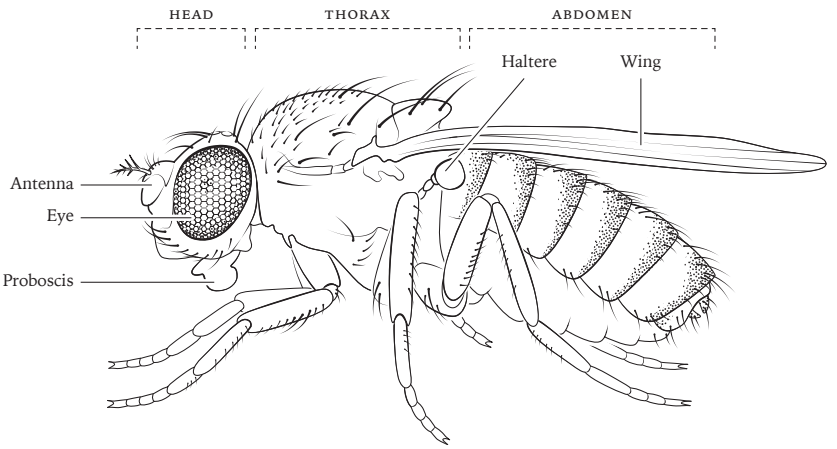
All this seems to beg the question, "Why?"

Why has this one tiny fly been the subject of intense scrutiny? And just as important, if we know so much already, why does research on the fly continue? After all, scientists now have access to the sequence not only of one person's genome but of thousands of them. Add to this, recent technical advances have made it possible to do meaningful, revealing experiments using human cells cultured in a liquid nutrient media. Researchers can also now experiment with cells taken directly from the normal or diseased tissue of patients suffering from specific diseases, and manipulate the sequence of genes within human cells with unprecedented precision. We can generate induced pluripotent stem cells (iPSCs) from cells scraped from skin or other tissues (Takahashi et al. 2007; Yu et al. 2007; Park et al. 2008), and "engineer" the DNA sequence of human cells, such as using the "clustered regularly interspaced short palindromic repeat" or CRISPR system (Jansen et al. 2002; Cong et al. 2013; Mali et al. 2013). Yet neither alone nor collectively have new advances in other systems supplanted or obviated the usefulness and relevance of *Drosophila* to biological and biomedical research. Every year many undergraduates, graduate students, and postdoctoral fellows join fly labs and initiate new fly projects, betting their careers on this red-eyed speck of a creature. Young and old, new to the field and established, fly researchers keep at it. Intrepid. Convinced. Loyal.

Surely this does not happen without substantial evidential support of the value of pursuing research with the fruit fly. There must be good reasons that thousands of highly trained, highly accomplished researchers keep studying the fly, as well as good reasons that national research funding agencies, private foundations, and nonprofit organizations—including organizations focused on specific human diseases—continue to provide grants to support fly-based studies.

True Flies, the Helicopters of the Insect World

From the perspective of entomology (the study of insects), a field centuries older than genetic fly research, *Drosophila melanogaster* has no particular



An adult female fly, *Drosophila melanogaster.* The body of an adult fly is organized into three main regions, the head, thorax, and abdomen. Like other true flies in the order Diptera, *Drosophila* have two wings (one pair) and a pair of halteres, drumstick-shaped structures at the base of the wing thought to help flies balance. Sensory bristles are present in a specific and regular pattern on most body parts, from head to tarsi (toes). Features of the head include the antennae, eyes, and proboscis (mouth).

appeal or importance. *Drosophila* is a "true fly" in the order Diptera, "di" meaning two and "ptera" meaning wing, the Greek word that gives us "pterodactyl." Unlike most winged insects, which have four wings (two pairs), true flies have two wings (one pair), plus a pair of specialized appendages called halteres, small ball-on-stick structures—shaped like drumsticks or maracas—located at the base of the wings. Halteres are an evolutionary specialization of the second pair of wings, often likened to a pair of gyroscopes, that make Dipterans some of the most skilled fliers in the insect world. True flies are more like hummingbirds in flight than they are like soaring vultures, more like helicopters than airplanes. They can dart about and they can hover.

Many insects with "fly" names are not true flies—butterflies, sawflies, mayflies, and dragonflies among them—and many true flies in the order Diptera do not have "fly" names—including mosquitoes, gnats, and midges. Although they are true flies, *Drosophila* are not "true fruit flies" as typically defined. In entomology, "fruit flies" is a term usually reserved for species in the family Tephritidae, which includes many notable crop pests, such as the infamous medfly or Mediterranean fly, and a slew of tiny beasts named for the crops they attack and destroy: the apple maggot fly, cherry fruit fly, peach

fruit fly, olive fruit fly, and so on. Unlike most flies in the family Drosophilidae, which includes flies in the genus *Drosophila*, Tephritidae lay their eggs in developing fruit or living plant material, and after they hatch, the larvae eat their way out, destroying or reducing the food value of the fruits or vegetables they consume.

As a result, Tephritidae are seen by many as enemies to mankind, whereas with the exception of the emerging crop pest *Drosophila suzukii,* flies in the family Drosophilidae are considered relatively innocuous, as they tend to be attracted to fruit only after it has ripened and begun to rot, or, in the case of some Drosophilidae species, are not attracted to fruit at all. In texts on agricultural pests written around the time *Drosophila melanogaster* became a favored research subject of biologists, *Drosophila* are not mentioned or are noted only as a nuisance: the 1912 edition of *Insect Pests of Farm and Orchard,* for example, omits mention of *Drosophila* altogether. Although an entry for "Drosophila" was added by another author in a subsequent edition, published in 1941, the discussion is limited to a small subsection on "Miscellaneous Pests of Food" that takes up less than a quarter of a page and has nothing more nasty to say about *Drosophila* than that they are "extremely numerous and annoying" (Peairs 1941). A nineteenth-century publication reportedly went so far as to suggest that *Drosophila* do us a service by spreading the yeasts necessary for fermentation of wine as they flit about in vineyards (discussed in Oldroyd 1965), an idea perhaps supported by much more recent findings related to the fly's sense of smell (Christiaens et al. 2014). Likely due to the meaningful differences between the impact of Tephritidae and Drosophilidae, fly biologists are sometimes scolded by entomologists for calling *D. melanogaster* "fruit flies" at all. Alternative names include the vinegar fly, the banana fly, the wine fly, and—using a term for the pulp left over after fruits like apples are pressed for juice—the pomace fly. (Following the current norm in biological and biomedical research publications and databases, from here forward, "fruit fly" or simply "fly," as well as *Drosophila,* will refer to *Drosophila melanogaster,* unless specified otherwise.)

Regarding the direct interaction of wild *Drosophila* with us humans, flies again hold no special place; they pose no discernible threat to us, living in our midst in relative peace without bite, sting, or prick. This is something that cannot be said of many of the fruit fly's cousins. The order Diptera includes a large number of flying monsters that do attack us, or attack the animals we rely on for food, companionship, and labor (Service 2012).

These include biting flies like horseflies, deerflies, and tsetse flies; smaller biting midges and no-see-ums; and the more than 3,500 species of mosquitoes (Service 2012). Even in the far reaches of the globe, biting flies and mosquitoes can make life miserable. An old pamphlet from the U.S. military on the problem of biting flies and mosquitoes in the Arctic, for example, notes that these blood-loving creatures "have been called the curse, the bane, and the scourge of the arctic summer" and suggests a number of steps that can be taken to protect oneself from them, including wearing two layers of clothing, light-colored socks tucked into boots, a "head-net" or brimmed hat draped with netting that covers the face and neck, and gloves that extend to the elbows (Arctic Desert and Tropic Information Center 1944).

These creatures are not just an annoyance. They can also act as "disease vectors," essentially injecting us with infectious pathogens when they bite or prick our skin to take a blood meal. The impact of Dipteran disease vectors on human health is profound. The mosquito *Anopheles gambiae* transmits *Plasmodium falciparum,* the single-celled eukaryote responsible for malaria, a disease that kills more than a half a million people each year. Other Dipterans spread viral diseases such as dengue fever, eastern equine encephalitis, West Nile fever, yellow fever, and Zika virus disease, which are transmitted by various types of mosquitoes; African trypanosomiasis or sleeping sickness, which is caused by the single-celled eukaryote *Trypanosoma brucei* and transmitted by the tsetse fly; and various forms of filariasis, which is caused by roundworms and spread by mosquitoes or biting flies (Service 2012). As a result, many communities work to control Dipteran disease vectors. In the United States, by the 1900s, the destruction of mosquitoes and their habitats was considered a civic duty, with eradication methods ranging from application of kerosene to standing water to the introduction of mosquito larva-eating fish such as sunfish, topminnows, and sticklebacks (Howard 1901). Many U.S. communities still have active mosquito control programs, sometimes carried out under broader municipal "vector control programs," which include the use of chemical insecticides and the introduction into standing water of biological controls such as the mosquito-toxic spores of a bacteria in the genus *Bacillus*.

Notably, not all Dipterans outside the family Drosophilidae are as nasty as the fruit-destroying true fruit flies or blood-feeding mosquitoes. Many types of flies contribute to the necessary decomposition of biomass, re-

turning nutrients to the soil. Others help pollinate flowers, much the way bees do. Still others are relevant as parasites that control population sizes of plants, animals, and other insects that might otherwise reach environmentally disruptive levels (Waldbauer 2003). In *The Families and Genera of North American Diptera* (1934), entomologist C. H. Curran went so far as to say that "No group of insects, except, perhaps, the Hymenoptera [bees, wasps, ants, and sawflies], are so important to mankind as are the flies. In these two groups are man's best friends among the insects. . . . If the world should suddenly find itself without flies and bees it would quickly revert to a sphere lacking animal and plant life, so important are these insects in maintaining the 'balance of nature' " (Curran and Alexander 1934).

Given the relatively innocuous relationship between *D. melanogaster* and mankind, these flies are clearly not justified as a research subject based on any kind of threat to our food or health. We are forced to look for other reasons why *Drosophila* has been the subject of sustained scrutiny. We might grasp for superlatives. Perhaps the fruit fly is the smallest, hardiest, most unusual, or rarest among its kind. But *Drosophila* do not meet the challenge. Are they the smallest flies? No. Fruit flies are small: at about 2.5 mm in length, they are less than half the length of a common housefly. But some other true flies are smaller. Some types of phorid or scuttle flies (family Phoridae), for example, measure just 0.5 mm in body length, making them five times smaller than *Drosophila*. Hardiest? No. *D. melanogaster* are considered cosmopolitan, living nearly everywhere that humans also live (Oldroyd 1965), but many of their cousins fare better in extreme conditions. Snow flies (family Limoniidae), for example, fill their blood with the natural antifreeze glycerol, allowing them to live at temperatures well below freezing, conditions *Drosophila* could not tolerate. At a different extreme, many species of flies and midges live in deserts or the seashore, where various specializations allow them to survive the baking sun and riling tides, also conditions in which *D. melanogaster* would not thrive.

Most unusual, then? Again, the answer is no. Fruit flies are not particularly strange looking, as are some other Dipterans, such as the alien-looking stalk-eyed flies, leggy crane flies, or wingless bat flies. As consumers of sugars and yeasts on rotting fruit, *Drosophila* do not have a particularly unappealing diet, as do many other types of flies, such as those that live on dung or carrion. *Drosophila* are not fierce predators like "assassin" or "robber" flies (family Asilidae), which can capture other insects in mid-flight, and they do not live in particularly unique settings, as do, for example, the

various types of gall midges (family Cecidomyiidae) that live inside plants, altering the very architecture around them by inducing the plants to form galls or knobby outgrowths; the carnid flies *Carnus hemapterus,* which seek out a bird's nest, shed their wings when they find one, and reproduce there; or the flies in the family Axymyiidae that live exclusively on wet rotting wood that is "pale, bare, waterlogged but not submerged" and "soft enough to penetrate with a jackknife but too hard to easily pry open" (Marshall 2006).

And as for rare, the answer is decidedly "No," as is evident to anyone who has ever put a ripe banana near an open window in the summertime (for a practical solution to this problem, see Appendix A). Yet for more than a century now, research on *D. melanogaster* has outpaced research on any other Dipteran—indeed, outpaced research on any other insect, large or small, strange or common, helpful or vile—and, with few exceptions, continues to outpace research on almost every other living creature on the planet.

And so the question remains, "Why?"

The Organism "Made to Order" for Genetic Research

Around the time the first white-eyed mutant fly was reported, in 1910, our understanding of how information was passed from one generation to the next was at an interesting intellectual pivot point. G. Mendel had published work in the 1860s demonstrating that specific "characters" were heritable, following specific rules based on the nature of the trait and passed along from generation to generation in predictable patterns. ("Characters" were also called "traits" or "factors" in early work and were later associated with "loci." For the most part, geneticists would now replace all these terms with "genes.") Mendel proposed that each of us gets one copy of each character from our mother and the other from our father, and suggested two "laws" governing inheritance of these characters. First, characters segregate, or behave as separate entities, rather than mixing. Generation after generation, Mendel's peas were either smooth or wrinkled, not blended or semi-wrinkled; similarly, colors of flowers were of one type or another, not blended shades. Second, Mendel proposed that characters sort independently, such that a plant that bears smooth peas is no more or less likely also to have flowers of a certain color. Moreover, he suggested that characters could behave in a "dominant" fashion, with one copy of that character

conferring a trait, or in a "recessive" fashion, requiring that both copies be affected in order to reveal the trait. Notably, despite the important conceptual advances offered by Mendel, the tangible mechanism by which inheritance of traits takes place—what physical entity is responsible for traits or for that matter, if there was a physical entity responsible—was not known.

As has been described by others, Mendel's now-famous work was largely ignored in his time but rediscovered around 1900, at which time it inspired related studies in a variety of organisms, including *Drosophila*. In the intervening years, several key observations had been made. By the time Mendel's work became an influence, researchers knew not only that organisms were made up of cells but also that cells contained chromosomes and that half the chromosomes were contributed by the father and half by the mother. Although it had originally been thought that all chromosomes were the same, by the time the first white-eyed fly was spotted in Morgan's lab, evidence was mounting that chromosomes within an organism were different from one another, and researchers were beginning to home in on the idea that chromosomes might contain the heritable information. Further exemplifying the state of the times, many terms we now take for granted were originally coined in the late nineteenth and early twentieth centuries, including the word "gene" itself, first used in 1909 (Sturtevant 1965).

It is in this context that *Drosophila* was propelled into use, most famously in a laboratory at Columbia University that became known as the "Fly Room" and in which many seminal studies were conducted (Kohler 1994), and later in laboratories all over the world. Genetic research is essentially a numbers game (Morgan 1964), with confidence in a given result increasing by bounds when a phenomenon observed to occur, say, in one of four animals is observed again in about 25 of 100 animals, 250 of 1,000 animals, and so on. Morgan's 1910 publication describing the pattern of inheritance of the white-eyed trait provides a concrete example of the power of fruit fly numbers: the publication, which describes experiments conducted over about one year, includes analysis of nearly 6,000 flies. Such a large number lends credence to the paper's conclusions and would simply not have been achievable in that time frame using animals such as chickens or mice, which take longer to mature and generate a considerably smaller number of offspring, or using a plant with a life cycle tied to the seasons.

Indeed, many attributes that make *Drosophila* a desirable research subject are centered at least in part around numbers. These attributes will sound familiar to many who have taken an introductory course in biology

or genetics: starting out, a researcher would be wise to choose a creature that is relatively easy to culture in a laboratory setting, such as one that does not require a lot of space or food; that develops rapidly, going from fertilized egg to fully formed adult in a relatively short time; and that is fecund and fertile, consistently producing a lot of offspring with each generation, even under laboratory conditions. Fruit flies fit the bill in all three categories: they can be easily cultured and maintained in small lab spaces; they develop from embryo to adult in about ten days; and they reproduce by the thousands.

Because in the wild *Drosophila* live on ripe fermenting fruit at both stages during which they feed (larval and adult stages), the early fly researchers needed nothing more than a ready supply of bananas to keep alive their fly "stocks" (individually cultured flies of specific genotypes) and set up new fly "crosses" (paired matings) between male and female flies of different genotypes. Moreover, compared to some other insect food mixes, both the original banana-based recipes and the more contemporary fruit fly food mixes, which are largely made up of corn meal and sugar, are less gross than what would have been needed to rear other types of insects for comparable studies. Foods recommended for rearing houseflies in large numbers, for example, include horse manure—obtained fresh, then pasteurized and mixed with yeast—and fermenting dog biscuits (West 1951).

Moreover, although the fly lifespan is considerably longer than one might think—under the right conditions, an adult fruit fly can live for a couple of months or more—the fly life cycle is conveniently short. A fly can start life as a fertilized egg on one day and, given warm, humid, summer-like conditions, within ten days that fly can get through larval stages, pupation, and metamorphosis to reach adulthood, at which point it can mate and produce its own offspring. Once it reaches adulthood, a single well-fed and healthy *Drosophila* female can lay on average roughly 25–50 eggs per day throughout her lifetime. With a lifespan of about two months, and the time to maturity of about ten days, under standard lab conditions a single mated pair of flies can be parents to scores of offspring in little more than a week, grandparents to hundreds in a month, and great-grandparents to thousands before their demise.

These characteristics of rearing *Drosophila* in lab settings compared favorably not just to other insects but also to other animals used in genetic studies in the 1900s, which included chickens, rats, and mice. These animals all take up more space, have longer life cycles, and produce far fewer

offspring compared with the fruit fly. When we add the fact that inherent to doing research in new areas is that there are no guarantees of success, the relative cost and risk of studying creatures that place greater demands on resources seem all the more apparent. A concrete example comes from an experiment performed early in the twentieth century by R. Pearl, a leading figure of that time who held a faculty position at Johns Hopkins University. Pearl reportedly performed a genetic experiment with chickens that extended more than 17 years and involved almost 5,000 hens—a study extending 16 years longer than it took for Morgan to observe, study, and publish the groundbreaking results of his 6,000-fly study of the *white* gene. Alas, in this case, Pearl's investment of time and resources was not productive: the results were later summarized as "without success" (Walter 1930). It is perhaps of note that some of the successful studies for which Pearl is best known were performed using *Drosophila*.

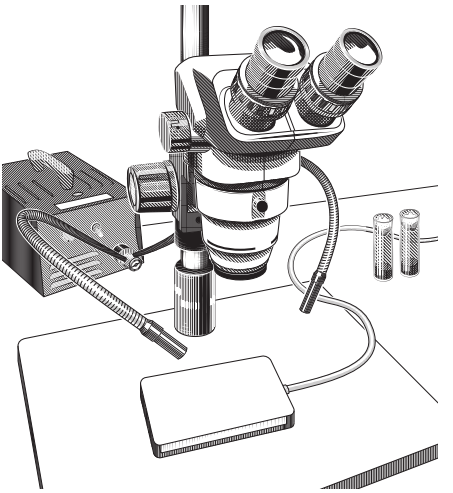
The relatively inexpensive cost of maintaining fly stocks and performing studies on large numbers of animals within a short period of time remains relevant today. The costs in staffing, lab space, equipment, and materials required to set up and run a dedicated "fly kitchen" that can provide enough fly food for the work of several average-sized labs, together with the cost of temperature-controlled incubators in which the flies are housed, is not insignificant. Nevertheless, they pale in comparison to the costs of running a facility for several other common model organisms, including the zebrafish and the mouse. Moreover, a typical fish or mouse facility in an academic research institution is likely to be maintaining far fewer animals than a typical fly group would be, in terms of both absolute numbers and the number of distinct genotypes maintained at the facility at any one time. Nevertheless, the total cost of running such a facility is far greater, requiring more staff, more equipment, and more space, and incurring more costs in day-to-day consumables than a fly lab.

Another boon to early fly researchers was that fruit flies have readily recognizable external features that turn out to be under genetic control, such that many mutant animals—including that first white-eyed fly—can easily be identified. For example, subsequent to isolation of *white* mutant flies, researchers went on to identify an artist's palette of mutant eye colors caused by disruption of one or the other of the two pigment-producing pathways. Like *white,* the genes were named for the eye colors of the mutant animals, giving us many early gene names still in use, including *brown, cardinal, cinnabar, prune, purple, rosy, ruby, scarlet, sepia,* and *vermillion.*

In addition to eye color, the early geneticists also detected mutations that affect the shape or texture of the eyes, giving us enduring gene names like *Lobe* and *glass*; the shape, length, venation, or straightness of the wings (*Notch, miniature, thickveins, Curly*); the color of the cuticle, the hardened outside of the fly, also called the exoskeleton or integument, which can be altered in color all over (*black, ebony, yellow*) or in distinct regions (*pentagon, speck*); and the number or shape of the sensory bristles present on the fly's body (*hairy, forked*). Indeed, the lengths and distribution of the bristles are so regular on a wildtype fly that comparison of the bristles in closely related species of flies has been used as a method for identification of distinct species, a practice known as "chaetotaxy," from the Greek *chaete* meaning hair and *taxis* meaning arrangement. It is also easy to distinguish male *Drosophila* from females. Males are smaller and more darkly pigmented at the posterior end; and have a strip of thick bristles called a "sex comb" on each foreleg, a feature absent in female flies. Male and female fruit flies also have readily observable differences in the shape and color of their external genitalia.

To examine flies, a contemporary fly researcher typically anesthetizes them by exposing them to carbon dioxide gas, which deprives them of oxygen. Exposure to ether vapor or to cold temperature is also effective to anesthetize flies, though less safe and controllable. Once anesthetized, flies can essentially be poured from a culture vial. With a few taps on the back end of the vial, anesthetized adult flies spill out onto a "fly pad," a device about the size of an index card that is made up of a layer of porous white plastic mounted on a half-inch high chamber that is connected to a source of carbon dioxide. The white color of the platform provides a convenient background for observing the flies' color, shape, and other external features, and the porousness of the platform allows the flies to be bathed in a steady stream of carbon dioxide, keeping them anesthetized during observation.

Once the flies are on a fly pad, the researcher can take a close look, moving the fly pad around under the binocular view of a "dissecting microscope," a relatively low-tech, low-magnification microscope commonly used in classrooms as well as in labs, to find and observe different flies, zooming in or out to get a better look at various features of individual flies. Researchers use small tools to move individual flies around on the fly pad—small paint brushes, carefully trimmed feathers, modified forceps, and tiny metal spatulas are common choices. At least by the 1930s these



A typical fly-pushing station. A microscope allows the researcher to view anesthetized flies on a white fly pad. Carbon dioxide gas is piped into the fly pad, providing an anesthetic. A pair of bright, directed sources of white light help researchers see the flies well under the microscope. A set of fly-pushing tools would be close at hand.

tools were referred to as "pushers," and the practice of observing, sorting, and sweeping anesthetized flies into vials became known as "fly pushing."

Using a microscope, a fly pad, and a pusher, a researcher might gently reposition an individual fly to get a close look at specific external features, or sort a group of flies based on the presence or absence of specific visible markers. This might be done to count how many flies are in each category, giving an idea of the pattern of inheritance, so as to ask whether flies of a specific genotype are under- or over-represented compared with a predicted outcome, or so that the researcher can set up a new cross by sweeping a fly or flies of a specific sex, and with specific marker phenotypes, off the fly pad and into a fresh food vial, where the flies will recover, move around again, and, when provided with a mate, go on to produce progeny. Watching a fly researcher sort, count, and push flies into fresh food vials is a little like watching a pharmacist sort, count, and push pills into a prescription bottle. Though it can take years to become an accomplished fly geneticist, fly pushing alone is not difficult. Given an appropriate microscope and a few pointers from an expert, most people can learn within a few minutes how to tell males from females, as well as how to identify a number of differences from the norm—"mutant phenotypes" different from normal

attributes of wildtype flies—that affect attributes like the shape or color of the eyes, or other external features.

In early studies using *Drosophila,* mutant flies in which external visible features were affected were a main focus; they were the main tools for discovery. Flies with various attributes (the parental or P_0 group) were crossed to one another, and the distributions of the external visible markers in their progeny (the F_1 generation), their progeny's progeny (the F_2), and so on, were carefully observed and counted. Subsequently, the same visible markers were used to follow other, nonvisible traits or mutations, with the visible markers serving as indirect indicators of what has or has not come along for the ride elsewhere on a chromosome. Whether they are the direct focus or simply indicators, visible markers can be used to select flies of a specific genotype from a mix of siblings of different genotypes. This in turn allows a researcher to set up very specific, controlled genetic tests. Some visible markers are much easier to "score" or distinguish from wildtype than others and, not surprisingly, the easy-to-score markers tend to get used more often. At this very moment, there is almost certainly a fly biologist somewhere who is separating white-eyed flies from red-eyed siblings, curly-winged flies from straight-winged siblings, or stubble-bristled flies from long-bristled siblings, in preparation for an experimental fly cross, dissection, DNA extraction, or other study.

In large part due to the fruit fly's ability to rapidly make more of itself, even in a laboratory setting, as well as the early availability of mutations affecting easily visible, external features, the results of research studies conducted with *Drosophila* contributed a tremendous amount of knowledge to our early understanding of genetics—knowledge pertinent to the roles of and relationship between genes and chromosomes, the rules and exceptions regarding patterns of inheritance, and the complex genetic interplay between parents and child. By the early 1920s, *Drosophila* was considered "a veritable bonanza to the geneticist" (Walter 1930). Results of studies performed using *Drosophila* lent further credibility to Mendel's theories on inheritance and helped reveal previously unknown or unproven relationships between genetic traits and chromosomes.

Over the next decade or two, the number of mutant *Drosophila* flies that had been isolated and cultured in labs went from one to a few to several, to hundreds, with the number exceeding 500 different mutant fly strains by the 1930s (Dunn 1934). Facilitated by technological advances, the number reached the thousands by the 1960s (Lindsley, Grell, and Bridges 1967)

and the tens of thousands thereafter. Given existing and new technologies, we now have exquisite control over genetic modification, and the number of mutant strains of *Drosophila melanogaster,* often touted as one of the most "genetically tractable" model organisms, is likely to increase further at a rapid rate as time goes on. Indeed, *Drosophila* is in the position of becoming the most complex multicellular organism for which we have isolated or engineered a mutation in every gene in its genome and determined the outcome of that perturbation, at least in broad strokes, such as whether or not mutation of the gene leads to death, sterility, or obvious changes to morphology under standard laboratory conditions.

Beyond Genetics

In a sense, for the purposes of the pioneering genetic studies, it would not have mattered if fly genes, and the RNAs and proteins they encode, were similar to or different from our own, so long as the way they are passed from generation to generation was the same. But as it turns out, the parallels between fly and human do not end with the mechanisms of inheritance. Far from it. The parallels extend much farther—to the makeup of specific genes; the structure and functions of the RNAs and proteins the genes encode; the controlled and interconnected ways in which sets of genes, RNAs, and proteins work together to bring about specific events or behaviors at the level of cells, tissues, organs and whole organisms; and the ways our various tissues and organs form, grow, communicate, repair damage, age over time, and respond to stimuli like infections, toxins, food, drugs, alcohol, heat, and other stresses. Evolution turns out to have been quite conservative and efficient in this sense. Once a problem was solved—say, once a critter evolved to have some particular gene or set of genes with a given function that proved useful—versions of that same solution were applied to new problems over and over again, both in different stages of life within one organism, and in solving similar problems as new organisms evolved. As a result of a shared evolutionary history, there are rich and meaningful commonalities between the sets of genes required to build and maintain a fly and those needed to build and maintain larger, more complex creatures, like us.

To look at a fly is to see, on the surface at least, a creature that seems quite unlike a human being. The adult fly has a typical insect-type organization, with a three-part body plan comprised of a head, a thorax, and an

abdomen, and with an external exoskeleton, rather than an internal skeleton as we have, providing structural support. Moreover, again common among insects, flies lay eggs, embryos hatch from the eggs as larvae, the larvae become pupae that undergo metamorphosis, and only then do the adult forms emerge. This mode of development is by all outward appearances quite unlike what we associate with human development, and the resulting adult fly looks very different from an adult human being. A somewhat deeper look, however, reveals more commonalities between flies and humans than are apparent at the surface. For example, like us, adult fruit flies have a complex brain, an internal clock, and five senses—sight, hearing, taste, smell, and touch—and share many types of tissues and organs with similarities to our own, in terms of both overall function and how they are formed during development. In addition to a brain, the fly has muscles, a pair of kidneys, a liver, a branched tubular network not unlike our lungs or blood vessels, a circulating fluid comparable to blood or lymph, a digestive system, and even a heart, complete with a rhythmic heartbeat (for a comprehensive list of human organs for which the adult fly has a comparable organ or cell type, see Appendix B).

In addition, the fruit fly brain is sophisticated enough to control a wide range of integrated behaviors—fruit flies can seek food; court mates with songs and dances; move away from predators; fight with one another over scarce resources; learn and remember; and maintain a diurnal pattern of circadian rhythms, awake and active in daylight and sleeping at night. There is good evidence that fruit flies can get drunk and that, once drunk, they become less discriminate in looking for mates (Wolf and Heberlein 2003; Lee et al. 2008), and that they can become addicted to drugs like cocaine (Kaun, Devineni, and Heberlein 2012). They can also "get," in most cases through genetic manipulation, any number of conditions with notable similarities to human diseases, including developmental disorders, cancer, diabetes, and neurodegenerative diseases such as Parkinson's disease. *Drosophila* are increasingly being used as "disease models" to uncover additional genes or therapeutic treatments that might ameliorate specific human diseases, and even as an emerging system for use in the new fields of personalized and precision medicine, which seem poised to create a new era in how we treat disease.

In short, although at first blush it might seem that comparing a human to a fruit fly would be similar to comparing a fully loaded, fresh-off-the-lot Lexus sedan to a toaster oven or a sewing machine, it turns out to be much

more like comparing that brand new Lexus to a 1976 Ford Mustang. Although the old Mustang might lack in sophistication, it does have commonalities in overall design and similar parts with similar functions, as well as commonalities in the way it was constructed, with what tools and what starting materials, and how it can be maintained. Moreover, again like the Lexus and the Mustang, the parts most similar between humans and fruit flies tend to be the parts that present the biggest problems when they fail—the equivalents of the starter motor, engine, gas tank, tires, chassis, steering wheel, and so on. Put another way, at a basic or fundamental level, the parts, assembly, and functions of the two systems are largely the same, even though many less critical features differ. In line with this idea, those of us using *Drosophila* and other model organisms in biological and biomedical research often refer to our efforts as "basic" or "fundamental" research, aimed at uncovering common truths applicable to broad groups of living organisms.

With all this in mind, it becomes easier to begin to understand the appeal of *Drosophila* research, both in the early going and today. To do research with the fly is to systematically uncover, in a relatively simple organism, some of the central themes that connect all or many living beings. Armed with new knowledge gained from the simpler form, researchers have an easier time approaching the study of much more complex creatures such as ourselves. Indeed, the idea that understanding the simpler form will help guide studies of the more complex is at the crux of what it means to do biological research with model organisms, including not just the fruit fly but also other subjects of contemporary genetic research, such as the yeast *Saccharomyces cerevisiae,* the nematode worm *Caenorhabditis elegans,* the zebrafish *Danio rerio,* the mouse *Mus musculus,* the small flowering plant *Arabidopsis thaliana,* and several other established and emerging model organisms. Moreover, many so-called "human" studies are performed using what are essentially a large variety of models rather than the real thing; that is, many studies of human gene function are performed using cultured human cells, often derived from cancerous tissue (as is true of the widely used HeLa cells), or in some cases generated through a series of molecular tricks to induce normal, final-state cells to take on a more naïve, dividing state—in either case, simpler systems than the whole.

None of us who use model organisms or cultured cells could compellingly argue that all aspects of human development, health, or disease could be modeled in any one of these systems alone, or even collectively. There

are limits to the utility of models, and always will be, given the straightforward fact that a yeast or a fruit fly or a HeLa cell is simply not the same as a human being. Similarly, there is a limit to the usefulness of *Drosophila* as a model of crop pests or insect disease vectors, again for the straightforward fact that a fruit fly is a fruit fly (or at least, a vinegar fly) and not a crop-damaging apple-maggot fly, disease-carrying mosquito, wood-eating termite, or any creature other than itself. By extension, and particularly in this age of rapid, affordable genome sequencing—biology's "big data" explosion—we see more clearly now than ever before that even within a species there are differences that manifest in meaningful ways, making each of us—or any individual of any species—a unique biological system, with its own strengths and weaknesses, its own set of internal signals and responses to environmental cues, and, as is increasingly argued, requiring its own personalized regimen of therapeutics.

Nevertheless, the parallels and commonalities between model organisms like the fruit fly and us humans, as well as between flies and other animals, are profound, varied, and fascinating. The results of more than a century of *Drosophila* research have revealed countless truths about the functions of our own genes and proteins, how we develop from fertilized egg to wholly formed being, how we age, what goes wrong in disease—the list goes on and on. Many unanswered questions can yet be addressed using model systems; continued research on *Drosophila* holds promise to reveal additional, profound new findings in the future, including but not limited to new insights into common and rare human diseases and new strategies for their treatment. Moreover, understanding how *Drosophila* research has contributed to biology and biomedical sciences can help us learn what it means to do research—help us see how research on smaller creatures contributes to an understanding of larger and more complex organisms—and help us begin to appreciate a few of the marvelous solutions to biological problems that evolved and endured, remaining largely the same in organisms as superficially different as the tiny flies and us humans.

CHAPTER ONE

MAPS

IT SEEMS A FAIR GENERALIZATION THAT PEOPLE LIKE MAPS. WE HAVE maps of neighborhoods, towns, cities, counties, regions, countries, continents, the entire planet, and beyond—maps of the lunar surface, the stars above us, the Milky Way, and the nether reaches of outer space. We have maps pertinent to a wide variety of features—maps of weather patterns, waterways, shopping malls, battlegrounds, butterfly migration, and meteor strikes. Fiction writers sometimes draw fictional maps of their fictional worlds, and these sometimes make it into the front matter of books. Robert Louis Stevenson created a map for *Treasure Island,* for example, and J. R. R. Tolkien for *The Hobbit*. We place value in maps for at least the straightforward reason that they can be exceptionally useful. Maps allow us to get a sense of places where we have not been (and perhaps will never go), navigate new places, and gain new understandings of familiar places. Importantly, they help us view as a whole a region that would be too large to depict at life size, as almost by definition maps present areas at an altered scale, with an inch corresponding to a mile, perhaps, or a centimeter to a kilometer. Different from photographs, maps also display features in abstract form, with some features left out and others represented in bold colors, bringing them to our attention. With maps in hand we can learn relative distances, find points of interest, distinguish roads from waterways, and

note changes over time. We can plan an excursion and navigate our route. We can know a place; have a more complete and connected sense of it; make tangible the far horizon.

Chromosomes, which are made of DNA, contain our genetic information, the coded instructions for making, maintaining, and reproducing the whole. As such, the chromosomes, or the genome they make up, are often referred to as the blueprint or instruction manual for a living thing. In a real and useful sense, however, chromosomes can also be thought of as landscapes—as geographical regions full of features, including but not limited to genes, each of which can be defined, annotated, diagrammed, and placed in meaningful relationship to other features along a chromosome's extent. In other words, chromosomes can be mapped. And just as we rely on maps to navigate, explore, and understand the landscapes around us, so, too, genetic maps (and now "genomic" maps) help us to navigate, explore, and understand chromosomes, altering the scale and highlighting subsets of features. They allow us to make sense of landscapes that are otherwise both inconceivably small and incredibly complex. But how do we make a "genetic" map of a chromosome?

The first genetic map ever made—the one that laid the foundation for how to make maps of chromosomes in other species, defining a method of cartography that went on to be used for a century—was not a genetic map of the chromosomes of rat, a mouse, a human, or even some simple, single-celled organism. The first genetic map—a geneticist's first rendering of the relative positions of genes—was a map of the *Drosophila* X chromosome. This feat, undertaken by a fly researcher who was an undergraduate at the time, was part of a larger body of work that revolutionized the field of genetics and forever changed our ability to study and understand genes.

Breaking Laws

When T. H. Morgan mated the white-eyed male fly with normal red-eyed females, then mated the offspring with each other, and counted how many flies had what color eyes in subsequent generations, he noted something surprising: the pattern of inheritance of the white trait—the numbers—were different for male flies than for females. In his 1910 report, Morgan begins by reporting the following data: "The F_1 hybrids, inbred, produced: 2,459 red-eyed females, 1,011 red-eyed males, 782 white-eyed males" (Morgan 1910). He then summarizes what was surprising about the results,

using italics for emphasis: "*No white-eyed females appeared*" among the grandchildren of the original white-eyed fly. Thus, not long after Mendel's laws had finally become widely discussed and accepted, a biologist was now presented with a notable exception. Mendel's law of independent assortment would suggest that there should be no relationship between two traits—in this case, eye color and sex. But right there in plain, irrefutable numbers was proof that these two seemingly unrelated traits were not sorting independently but instead were "linked" together by a shared pattern of inheritance.

Proof in the form of thousands of flies was likely important to acceptance of the data and its interpretation. Only a year before, W. Bateson had stated that "Of the various cases alleged as exceptional, or declared to be incompatible with Mendelian principles, few have any authenticity" (Bateson 1909). On the other hand, a few other exceptions to the rule of independent assortment had already been noted. In 1900, C. Correns reported that for one type of flowering plant, white-flowered plants invariably also had hairy leaves and stems, whereas red-flowered plants had smooth ones, providing the first example of "complete linkage," wherein two seemingly independent traits always appear together. In 1905, Bateson and R. C. Punnett, also working in plants, reported "incomplete linkage" (now referred to simply as "linkage"), wherein a pair of traits are more likely to be seen together in the same offspring than Mendel's law of independent segregation would predict, but, unlike with complete linkage, do not always appear together (Sturtevant 1965). By this time, researchers had begun to realize, including through work in *Drosophila* by N. Stevens, that the X and Y chromosomes are special, defining who will be female and who will be male (Brush 1978). What was different about Morgan's result was the connection between a trait like eye color and this trait of sex associated with physical structures found within cells.

Morgan sought to explain his results linking eye color with the sex of the flies as follows. He proposed that the male is heterozygous for the sex chromosomes (we would now describe this as XY in genotype), whereas the female is homozygous (XX). He further suggested that there is a "coupling" of the "red-eyed factor" with the X chromosome. We would phrase this differently now, describing the *white* gene not as "coupled to" the X chromosome but as located on it. But this is just semantics. Morgan was right. Aided by the fact that he could observe a large number of flies of multiple generations in a relatively short period of time, Morgan had

uncovered and demonstrated the phenomenon known as "sex linkage," the fact that some genes are "linked to" or present on the X chromosome and absent from the Y chromosome, such that any trait or mutation on the X is likely to be linked to sex. Since female flies have two copies of the X chromosome (one from the mother, the other from the father) and males have only one copy (from the mother, as well as a Y chromosome from the father), males are vulnerable to a mutation in their single X chromosome that would typically be masked in females by the presence of another, normal copy. In the case of white-eyed males, the single copy of the X chromosome that carried a mutation in the *white* gene was out there on its own and not supplemented by a second, normal copy, as would be true in a female heterozygous for the *white* mutation (Morgan 1910).

Finding Linkage

Morgan famously surrounded himself with a group of talented undergraduates, graduate students, and other researchers. This Fly Room group identified a number of additional mutant fly strains with visible defects or phenotypes affecting the eyes, wings, bristles, cuticle, and so on, as well as mutations resulting in lethality or sterility. As the team isolated additional mutant fly strains, they were able to uncover new relationships among them. In addition to recognizing that the *white* gene was linked to the X chromosome, the early *Drosophila* researchers found that although some genes, like *white,* were sex linked, others were not linked to sex but did behave as if "linked" to one another—they were more likely to appear together in a next generation, here again breaking Mendel's law of independent assortment. Eventually it was proposed that "closely linked" mutant genes, those that most often appeared together, are physically close to one another; that some genes are located on the same chromosome as one another, and along that chromosome, some genes are closer together than others. In practice, researchers would mate flies with one trait (say, *white* mutant flies) with flies with traits of another (say, flies mutant for *yellow* or *speck*). They would then do additional crosses among the offspring, revealing the frequency with which the mutations stayed separate or unlinked, as fits with Mendel's law, or traveled together, behaving as linked traits.

After careful testing of many combinations of mutations, the early Drosophilists narrowed things down to four "linkage groups," which we now know correspond to the X chromosome and the fly's three "autosomes,"

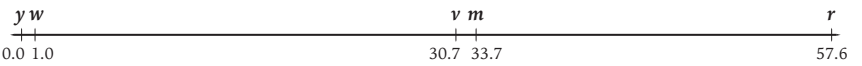
that is, chromosomes other than the sex chromosomes. With the X chromosome the implicit "chromosome one," the other three were named chromosomes two, three, and four. The finding that *Drosophila* have just four chromosomes (plus the Y) provides another example of the relative simplicity that makes the fly a premier organism for genetic research. The fact that fruit flies have fewer chromosomes than we do (four pairs versus our twenty-three pairs) is not simply a function of their smaller size. Even among insects, the number of chromosomes varies widely. Silkworm moths, for example, have twenty-seven pairs of chromosomes and some other true flies have six pairs.

Despite their advances in relating conceptual "traits" with physical chromosomes through the phenomenon of linkage, the Fly Room team was faced with a remaining problem: how does one relate or associate the numerical data they were accumulating regarding linkage to the physical world of chromosomes? A student working in Morgan's lab, A. H. Sturtevant, came up with a solution.

The First Genetic Map

Like Mendel's law of independent assortment, the phenomenon of linkage had an inherent set of rules. One of these is that the frequency of observing traits independently, as opposed to together, remains constant for any pair of traits. For example, the frequency of seeing mutant forms of *yellow* and *white* separated from one another, rather than linked, remained the same over multiple tests, as did the frequency of seeing *yellow* and *vermillion* separated. However, the frequency itself was different for any pair of genes; separation of *yellow* from *white* is a much less frequent event than separation of *yellow* from *vermillion*.

Morgan and his team, including Sturtevant, collected a large amount of data on linkage, determining the frequency with which traits became unlinked for as many X-chromosome and other traits as they could test. What Sturtevant did that was so important was to recognize that the numbers they were generating could be interpreted as relative physical distances. Sturtevant later described this key moment of insight in his 1965 book *A History of Genetics* (Sturtevant 1965): "I suddenly realized that the variations in strength of linkage . . . offered the possibility of determining sequences in the linear dimension of a chromosome. I went home and spent most of the night (to the neglect of my undergraduate homework) in producing the

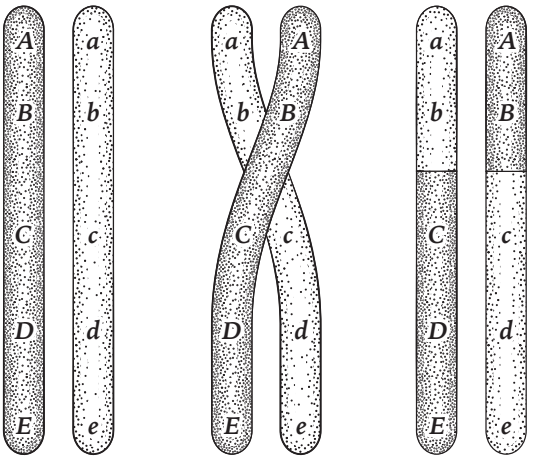


The first genetic map. Units in centiMorgans (cM). The spacing here is the same as in Sturtevant's original genetic map of the Drosophila X chromosome but the symbols reflect the contemporary nomenclature. The full gene names are *y, yellow*; *w, white*; *v, vermillion; m, miniature; r, rudimentary.* Modified from A. H. Sturtevant (1913), "The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association," *Journal of Experimental Embryology* 14 (1): 43–59, Diagram 1.

first chromosome map," which represented the relative positions of the X-chromosome genes *yellow, white, vermillion, miniature,* and *rudimentary.* The original map, published in 1913, is modest. It consists of a straight line annotated with five tick marks and corresponding lettered labels, representing the positions of the genes along that line. The key pieces of information are the order in which the tick marks appear and the relative spacing between any pair of them. The units of distance proposed for the map were not absolute like inches or millimeters, nor were they based on the counting units of chromosomal DNA, the individual "bases" or units A, C, G, and T; that level of detail regarding the makeup of chromosomes would not be uncovered for some time to come. Instead, Sturtevant expressed the values as percentages of the total length of a chromosome. He named these counting units "Morgans" in honor of his advisor and divided them into smaller units, "centiMorgans" (abbreviated cM), which became the commonly used term.

In creating this system, Sturtevant provided the world of genetics with the first meaningful representation of the distances separating genes from one another along chromosomes—the first genetic map. It was an approach that could be applied in other organisms, and one that proved accurate when researchers later applied new methods to building maps of the chromosome landscape, including DNA sequencing. Looking back, Sturtevant wrote in 1965 that the genes were arranged on that first map "in the order and approximately the relative spacing that they still appear" on maps at that time (Sturtevant 1965). More than one hundred years after the original map was drawn, and in an era in which we now know the precise distances between genes like *yellow* and *white,* down to the level of single units of DNA, the original 1913 map still looks pretty good.

Eventually geneticists would be able to attribute the phenomenon of linkage to physical structures and phenomena related to the chromosomes.



Chromosome "crossing over" as envisioned by early twentieth-century geneticists. Crossing over, which occurs during meiosis, explains "incomplete linkage" as observed by Morgan, Sturtevant, and colleagues. Usually, nearby versions of genes (indicated as A, B, C, etc.) remain on one chromosome, traveling together, such that the traits they confer appear to be "linked." Sometimes, however, chromosomes "cross over" or entwine, requiring an untangling process that can exchange part of one chromosome for another. The larger distance between genes A and E provides a greater chance that a crossover between them will occur, explaining the relationship between linkage and physical distance along a chromosome.

During production of the sperm or egg (the germ cells), the two chromosomes of a pair, one originally inherited from the organism's mother and the other from the father, align. Moreover, on occasion, these aligned maternal and paternal chromosomes get stuck together. Because the chromosomes are separated to form the final haploid sperm or egg, such a tangle must be resolved. Sometimes the separation following this "crossing over" leaves the originals intact, and sometimes recombination occurs, wherein the maternal and paternal regions of a chromosome are physically exchanged, with a bit of the maternal chromosome replacing the equivalent bit on the paternal copy of that chromosome, and vice versa. Thus, linkage could be used to draw a map because the larger the distance between a pair of genes or traits, the greater the chance that a cross-over would occur between them, resulting in a larger proportion of offspring in which a pair of traits became separated.

Good roadmaps provide an immediate, accurate sense of the relative distances of key destinations and features. The early genetic maps were useful, and became only more detailed and accurate over time as more mutant

strains were identified and the results of crosses to other genotypes were carefully counted, determining with precision the rates of recombination between any given pair of traits. Over tens of years, fly researchers would go on to isolate more traits in more mutant strains. The routine first step researchers performed with newly generated mutations, from the 1910s to the present day, was to "map" them to a chromosome and then to a specific region within it. For *Drosophila* specifically, Sturtevant's single straight line with five tick marks and labels was followed by much more extensive maps, comprising a set of four lines of different lengths representing the relative sizes of the X, second, third, and tiny fourth chromosome, each with its own set of precisely positioned tick marks and labels, corresponding to newly mapped mutations.

The impact of genetic mapping cannot be overstated. In a 2015 essay, biologist S. Henikoff wrote that although the field of genetics "was born with Gregor Mendel's classic paper . . . it was A. H. Sturtevant's introduction of the first genetic map in 1913 that defined the field of genetics as we know it today" (Henikoff 2015).

What is a "Gene"?

We might expect that a "gene" is now something easily defined, with a fixed start and finish along a chromosome. But this is not the case. Ask a biologist exactly where a given gene starts and ends, and you might find that the biologist, even an expert in genetics, struggles to answer. We can first remember that most genes encode proteins: gene sequences are first "transcribed" into RNA, as one might make copies of a single page of a book, and then the RNA molecule is "translated" into the language of amino acids to form a protein. The protein-coding region of a gene is typically easier to define than other regions—protein-coding regions follow the rules of the three base-pair genetic code, beginning with a three-letter "start" sequence and ending with a "stop," with stretches of three-letter "codons" of varying lengths between the stop and start encoding the specific amino acids that define a given protein. Other regions of a gene that researchers seek to define include regions of DNA that encourage or discourage the transcriptional machinery to hop on and start making RNA, termed "enhancers" and "suppressors," respectively, and the positions of splice sites separating "introns" or intervening sequences from "exons" that will be preserved in the mature RNA transcript. Some genes do not encode

proteins, but instead encode RNAs that function directly in their unmodified, transcribed form, or are further processed into an active form, in either case never translated into a protein. These "noncoding" or "functional" RNAs play structural, regulatory, and enzymatic roles, acting alone or in association with proteins or DNA.

In many cases a gene is transcribed to form a single RNA sequence that encodes just one protein. However, there are three places along the pipeline from DNA to RNA to protein that can result in a single gene encoding more than one RNA and protein. First, in some cases, transcription of the gene into RNA can begin at more than one starting position (that is, at more than one "transcription start site" or TSS), such that the same gene can encode two or more different versions of the RNA, each with a different leader sequence. Some of these RNAs encode proteins that will, correspondingly, differ at their starting ends. Second, RNA molecules can be "spliced" or cut and reconnected in different ways, such that the set of RNAs that might be made from one gene, and thus the proteins encoded, is greater than one, even for genes with only one TSS. Notably, information pointing to what sites along the RNA should be spliced is encoded in the DNA; RNA splicing is a very specific and controlled event, not a random reshuffling. Finally, once made, proteins can be "cleaved" (cut) into two or more smaller pieces, or modified by the addition of some accessory such as a phosphate group, a lipid molecule, or a sugar chain that adds further diversity. These "post-translationally modified" versions of proteins can have different functions and be localized to different regions within the cell, or both, as compared with the "uncleaved" or unmodified form.

We can place the events of "gene expression," the production of RNAs or proteins, into the physical context of the cell. Transcription and splicing take place in the nucleus; translation occurs in the cytoplasm. Post-translational modifications such as phosphorylation or cleavage can occur in any of many subregions within the cell, or after a protein has left the cell. Once made, proteins and functional RNAs relocate to disparate subregions of the cell—as dwellers of an apartment complex leave to go to their various jobs. The workplace of a protein or RNA might be back in the nucleus, in the cytoplasm, or at the cell surface, or involve shuttling back and forth between two or more locations. Other proteins might relocate, once made, to the inside of a subcellular organelle, such as the mitochondria or lysosome; be secreted outside the cell and stay close by, as is true for proteins that form the "extracellular matrix," the mortar between cells; or leave

and travel far from the cell in which they were made, as is the true for proteins that communicate information at long range between one organ and another.

Seeing Chromosomes, Making Connections

A key aspect of sex linkage as observed for the *white* gene was the intellectual link it formed between the concept of inheritance and a specific physical structure that biologists of the day had observed under microscopes: the chromosomes. N. Stevens and E. B. Wilson can be credited with providing evidence in 1905 that the Y chromosome is associated with maleness, providing a link between chromosomes and traits (Brush 1978). C. Bridges, another member of Morgan's Fly Room group, is lauded as providing definitive proof of an association between chromosomes as physical structures and the inheritance of traits. A peculiar aspect of the cell biology of the fly salivary gland turns out to play a role. Insects, including *Drosophila,* have specialized chromosomes in some of their cells, including the salivary gland cells, in which the DNA has been copied over and over without cell division. These chromosomes are aligned with one another like so many pencils in a box. Subregions along a single chromosome were not visible using the technology of the day. Nevertheless, subregions of "polytene" or highly amplified and aligned chromosomes were visible when they were removed from cells, mounted on glass slides, and stained with a purple-blue dye called Giemsa (Wilson 1907). How can we see features in polytene chromosomes not visible along a single chromosome? Imagine how much easier it would be to recognize from afar that pencils have in common a bright metal crimp, always appearing after the yellow shaft and before the pink eraser, when those pencils are lined up in a box, the aluminum appearing as continuous shiny band, as compared with viewing a single pencil from the same distance. Different chromosomes have different patterns, with one polytene chromosome like a box of pencils, another like a box of ballpoint pens, such that the "banding pattern" of each can be distinguished from the others.

Polytene chromosomes had been observed in cells of other insects prior to the era of *Drosophila* research, including by the nineteenth-century scientist E. G. Balbiani, who in 1881 noted their presence in the midge *Chironomus* (Balbiani 1881). In the 1910s, Bridges took the analysis of insect polytene chromosomes to another level, both by making painstaking repre-

sentations of the banding patterns of *Drosophila* polytene chromosomes and by looking at the banding patterns in special genetic situations, such as when different chromosomes had become physically connected to one another, a phenomenon known as "nondisjunction." By determining the specific banding pattern of the polytene chromosomes (that is, figuring out, based on their patterns, which chromosomes are attached to one another) and then matching these up with phenotypic outcomes, including what sex the flies appeared to be, Bridges was able to provide, in his words, "direct proof" that chromosomes are the physical structures that carry the encoded genetic information (Bridges 1914).

The availability of polytene chromosome maps added a new level of detail to the classical genetic maps. The polytene maps helped to make genes and chromosomes tangible, physical, something seen. The specific patterns of darkly stained bands and light regions or "interbands" of polytene chromosomes later served to inform other studies, contributing to our ability to determine the DNA sequence of specific genes and revealing information about gene expression. It is through observation of changes in the normal polytene chromosome banding patterns, for example, that in the 1960s F. Ritossa uncovered the first evidence of a "heat shock response," or the turning on of specific sets of genes in response to a stress such as heat (Ritossa 1996). Moreover, researchers have used polytene chromosomes to examine other aspects of chromosome conformation or rearrangements and their impact, taking advantage of what they could readily observe in the polytene chromosomes and then associating these observations with specific mutant phenotypes. As we sequence more and more human genomes, we gain a new appreciation for chromosome organization, as well as the ramifications of rearrangements, duplications, or deletions of large segments of DNA, phenomena long ago observed in polytene chromosomes and extensively studied in *Drosophila*. Indeed, studies in flies laid some of the groundwork for our current understanding of chromosome organization, including disruptions of organization associated with inherited disorders.

Bridges's work on nondisjunction has been praised by many, including by Morgan, who wrote that the theory developed via studies of chromosome nondisjunction "has been found to be fundamental for genetic interpretation of the action of genes" (Bridges and Brehme 1944). Contemporary biology takes for granted the idea that chromosomes are physical structures carrying encoded genetic information in the form of DNA. It is difficult now

to grasp how important an advance this was at the time. Bridges's detailed representations of the polytene chromosomes (Bridges 1935) have remained a resource for *Drosophila* geneticists ever since. They provided an important alternative map—one based on physical structures—that supplemented genetic maps based on Sturtevant's method. A lucky few mutations could initially be mapped to specific locations along the polytene chromosomes because they were associated with deletions or inversions of the DNA so large that a difference in the pattern of bands in the polytene chromosomes was visible, even using the limited microscopy methods of the day. Later, when a method of direct labeling of DNA or RNA called "*in situ* hybridization" made it possible to match up a small piece of DNA with its location on polytene chromosomes (Gall 2015), researchers were able to determine the locations of many more genes along this physical map.

Altogether, the work of Morgan and others in his group had lasting impact. One geneticist stated in the 1930s that from Morgan's work with *white* and the studies that followed "grew the general idea that all of the genes which determine the characters of the organism are located in the chromosomes; that each gene has a definite location in a specific chromosome and that the laws governing the distribution of the genes are actually the laws governing the distribution of the chromosomes" (Dunn 1934). The work was recognized in 1933 with a Nobel Prize to Morgan "for his discoveries concerning the role played by the chromosome in heredity" (Nobel Media 2016).

A Culture of Sharing

Catalyzing progress, members of the growing, early twentieth-century fly genetics research community were committed to openly sharing their mutant fly strains, protocols, and data. Despite fierce competition for jobs, grants, and other resources, the culture of sharing still largely sets the tone in the contemporary *Drosophila* research community (Rubin 2015). Morgan and his team published lists of their mutant fly stocks and made them available to colleagues. In the 1930s, fly researchers founded a newsletter, *Drosophila* Information Service (abbreviated DIS), which was meant to supplement the type of research reports that appeared in the scientific literature with timely information and practical advice, as well as mutant fly stock lists. By the 1940s, DIS could no longer handle the large volume of reports of new mutant fly stocks it received. To keep up with need, *Dro-*

sophila researchers produced a reference book, a de facto dictionary of fly genes and their mutant phenotypes, which was periodically updated. The book became known in the field as the "red book," based on the red covers common to later editions (Lindsley, Grell, and Bridges 1967; Lindsley, Zimm, and Lindsley 1992; Bridges and Brehme 1944).

Eventually, the volume of new *Drosophila* gene information and the pace at which it was being collected outgrew the book format as well. In 1989, W. M. Gelbart, T. Kaufman, K. Matthews, and colleagues established FlyBase, an online database of fly gene information (Ashburner and Drysdale 1994; FlyBase Consortium 1994). It was an early time for biologists to be thinking of such things; the original version of FlyBase was an FTP site accessed via Gopher rather than HTTP (FlyBase Consortium 1994). FlyBase became an essential resource for *Drosophila* biologists, as well as a model for how to establish and organize similar databases for other model systems useful for sharing gene and mutation information and, later, genomic data. Further emphasizing the fly community's long-time commitment to sharing, when maintaining and sharing fly stocks with colleagues became a burden for individual labs, stock centers were established for centralized maintenance and distribution. The collegial culture of *Drosophila* research helped to sustain fly research through tough times, as exemplified by updates shared in DIS by labs in war-torn Europe in the late 1930s and early 1940s, and continues to be an influence, and source of envy, for other research communities. Moreover, this sharing culture facilitated efforts to sequence the full fly genome and quickly disseminate information gained from that endeavor.

The First "Chromosome Walk"

Knowing the location of a gene is one thing; knowing the DNA sequence of the gene—giving us an idea of what functional RNA or protein sequence the gene encodes—is another important advance. It can be difficult to grasp the exceptional thinking and hard work that, in the pre-genome era, went into uncovering the identity and sequence of genes and in particular how researchers were able to match up a mutant phenotype with a gene, and, then with a specific DNA change (or changes) in that gene. Today, biologists working in common model systems such as the fly take for granted the possibility that once a mutation has been identified, they can focus in on the physical material of chromosomes, the DNA itself and its sequence,

normal or altered. This was, of course, not always the case. In the early days of molecular biology, segments of DNA could be isolated and copied, even reintroduced into flies, testing for its ability to reconstitute some activity absent in a given mutant strain. However, although polytene chromosomes gave us a distance view of the terrain, it was not a view that provided information about specific DNA base-pair changes. When it came to polytene chromosomes, we were instead like persons atop a high mountain who, without the benefit of binoculars or zoom lens cameras, had little hope of spotting a tulip bloom in the valley or the single brown leaf on an otherwise healthy tree on a distant hill.

In 1983, more than fifteen years before the full genome sequence of fly or human genomes would become available, P. Spierer, A. Spierer, W. Bender, and D. Hogness (Spierer et al. 1983) reported the development of a method that assisted in matching up the DNA sequence of a gene with a mutant phenotype: chromosome walking. This technique, another example of something applied first in the fly and then adapted for use in other systems, gave researchers a molecular foothold, making it possible to approach a gene from the side, take it by surprise, as it were, and correlate a fragment of DNA with a specific mutation. With a chromosome walk, a piece of DNA thought to be near the gene region of interest is used as bait to fish for additional fragments of DNA that extend out in one direction or the other. Molecular and genetic tricks are then used to determine whether things are headed in the right direction, as well as when the walk has extended far enough—and has reached the gene. The sequence of that fragment of DNA can then be determined, including a comparison of the wildtype and the mutant sequence, and an assessment of their likely impact on protein sequence.

Notably, the chromosome walking approach developed by Hogness and colleagues was quickly applied to the identification of mutations associated with human diseases; its application in the cloning of the gene that is altered in patients with cystic fibrosis, for example, is dubbed by some the first "important" use of the chromosome walking method. Indeed, in the "race to find the gene" that is altered in patients with cystic fibrosis (Merz 1989), chromosome walking and a related approach called chromosome jumping were instrumental in narrowing the gene region from a distance of about 1.5 million base-pairs of DNA, an unapproachable size given the DNA sequence technologies of the day, to a much smaller distance. This made it feasible to determine the DNA sequence of normal and disease-associated

forms of the human "cystic fibrosis transmembrane conductance regulator" or *CFTR* gene, which turned out to be about 100,000 base-pairs in size (Porteous and Dorin 1991). Groups led by R. Williamson, L. C. Tsui, and F. S. Collins (who went on to lead the Human Genome Project and in 2009 became head of the U.S. National Institutes of Health) each contributed information that led to the identification and DNA sequencing of *CFTR* (Schmiegelow et al. 1986; Eiberg et al. 1985; Rommens et al. 1989).

Researchers hoped that upon learning the mutant sequence of the normal and disease-associated copies of the *CFTR* gene, a cure for cystic fibrosis would become evident. The hard lesson of the time, and perhaps of the Human Genome Project more generally, was that this is, sadly, not typically the case. Learning what gene is affected in an inherited disorder, or even learning the specific DNA changes associated with the disease, does not magically lead to a cure (Cardon and Harris 2016). It does have a positive impact, however. Finding the causative gene can help define what disease a patient has, giving families some sense of what to expect and making connections among cases that might otherwise be missed, and can, in the long term, help researchers develop therapeutic strategies. A way to "fix" the mutation would seem ideal but has proved elusive. The CRISPR system, a molecular tool developed based on a natural defense mechanism of bacteria against viral invaders (Jinek et al. 2012), provides a new glimmer of hope, as this system offers a potential new route to making corrective changes to DNA sequences in human patients (Savic and Schwank 2016). Among the significant barriers to the approach, however, are ensuring an "on-target" effect (that is, modification only of the intended DNA sequence and not of other sequences in the genome) and the safe delivery of components of the gene editing system into cells (Tsai and Joung 2016; Savic and Schwank 2016).

From Roadmap to Satellite Imagery

Although important in their time, the early genetic maps of the fly chromosomes look rudimentary to us now. Hundreds of genes were mapped in the first half of the twentieth century, but this turns out to be a small fraction of the total number of genes present along the fly's four chromosomes. New information is often obtained via the invention of new technologies. Deep insights into the nature of genes and chromosomes followed from the identification of DNA structure, breaking of the trinucleotide DNA code, and

development of methods for sequencing DNA. Biologists today have at their disposal full-genome sequence data for humans, all common genetic model organisms, and many more species, as well as a good understanding, at least in some systems, of the extents and limits of specific genes along these sequences; that is, good "annotation" of protein-coding regions and other features along the genome sequence. This availability of a well-annotated, full-genome sequence is, however, relatively recent. The first genome sequences were gained through many years of systematic work, and facilitated by a series of advancements in DNA sequencing technologies. These advances are comparable to the technological developments that took us, over a similar period of time, from first-generation, monochrome personal computers to much more powerful, full-color computers, tablets, and smart phones.

Concurrent with the race to complete the human genome, researchers were also working hard to complete the full-genome sequences of common model systems, including *Drosophila*. Scientists rightly assumed that learning to sequence and computationally "assemble" the small bits of contiguous sequence that came off the sequencing machines into continuous chromosome lengths for a simpler organism would inform similar work with human genome sequence data. One advantage of trying things out in *Drosophila* was that in this species, researchers could benchmark the new sequence data against what was known already from genetic and polytene chromosome maps. The first full-genome sequences of any species were for viral and bacterial genome sequences. As they are orders of magnitude smaller than the genomes of eukaryotes, the genomes of these microbes were easier to sequence and assemble. Eventually, large-scale efforts and technological innovations converged, and larger genome sequences were completed. The first full-genome sequence of a eukaryote was that of the yeast *Saccharomyces cerevisiae,* published in 1996 (Goffeau et al. 1996), and the first full-genome sequence of a multicellular eukaryote was that of the nematode worm *C. elegans,* published in 1998 (C. elegans Sequencing Consortium 1998). The full-genome sequence of *Drosophila* came close on the heels of these other successes. Completion of the fly genome sequence was made possible in part by a collaboration between fly researchers and Celera Genomics, which was interested in using the fly genome sequence data to test a new computational approach to genome sequence assembly. The approach was a success. A first "draft" of the *D. melanogaster* genome sequence was published to much fanfare in March 2000 (Adams et al. 2000). The first human genome sequence followed a year later, in 2001.

These notable and much-publicized successes, together with improvements to sequencing technology and sequence data analysis, harkened the dawning of a new age in biological and biomedical research, alternately called, with the same meaning in mind, the "genomic era" or the "post-genomic era" (the latter terminology appears to have won out over time). Though claims like first eukaryotic or first multicellular organism were already taken when the fly sequence was released, the *Drosophila* genome can claim status as the first full-genome sequence of a creature with eyes and legs. That first *D. melanogaster* genome sequence, referred to as the "reference" genome sequence to distinguish it from the full-genome sequence of other strains of the same species, has since been updated, with new "releases" of the assembled genome sequence numbered like software versions—1.0, 2.0, and so on, reaching 6.0 by 2015 (Matthews et al. 2015; Crosby et al. 2015). Though most of the genome sequence was covered by 2000, the new data releases have helped fill in gaps. Like the gradual filling in of accurate details regarding the far northern territories on early maps of North America, additional work has helped resolve the sequence of previously sketchy regions of the *Drosophila* genome, including regions of the Y chromosome, which became easier to sequence and annotate as technologies advanced (dos Santos et al. 2015).

One of the surprises of the *Drosophila* genome sequence, as well as of the human sequence (Moraes and Goes 2016), was the relatively small number of genes identified in the full genome sequence. Although greater than the number estimated by some (Garcia-Bellido and Ripoll 1978), the number of genes in the fly genome was fewer than many researchers had predicted. The *Drosophila* genome is now thought to contain about 14,000 protein-coding genes (about 18,000 in total) (Kaufman 2017). The human genome, which similarly turned out to contain fewer protein-coding genes than expected, is thought to encode about 25,000. The presence of 14,000 protein-coding genes in the fly genome, however, does not mean that the fly genome has the ability to produce only 14,000 unique proteins (nor the human genome only 25,000). As mentioned previously, one gene can result in the production of more than one unique RNA and protein, with the "isoforms" or different proteins produced by a gene differing by a few amino acids or much larger stretches, and further subject to cleavage and post-translational modifications. The current estimate is that the 14,000 protein-coding genes can encode about 22,000 different proteins (Kaufman 2017).

Altogether, our understanding of the meaning of the full-genome sequence of *Drosophila* and other organisms, including humans, has expanded

since 2000 (Moraes and Goes 2016; Brown and Celniker 2015). Through efforts such as FlyAtlas (Robinson et al. 2013; Chintapalli, Wang, and Dow 2007) and the large-scale and multi-institutional collaborations that took part under the umbrella of the U.S. National Institutes of Health's Encyclopedia of DNA Elements (ENCODE) project (modENCODE Consortium et al. 2010), we now have an unprecedented view of which *Drosophila* genes are expressed at what developmental stages (that is, in embryos, larvae, pupae, or adults) and in what tissues or organs. At a practical level, whenever a fly gene is newly implicated in a given activity or process under study, we can now go to a database and get a quick sense of when and where the gene is expressed, making RNA. This information can help guide further study, in some cases suggesting in what cell types the gene is required and in what processes it might be involved. Combining microfluidics with next-generation sequencing, researchers are now able to ask what genes are on and off in individual cells. With so much information at our fingertips, the cycle of discovery, hypothesis, and experimentation, which leads to additional new discoveries and refinement of previous understandings, has become accelerated and focused. Existing datasets contribute to our interpretation of new findings, help us home in more quickly on the right set of experiments to do next, and give us answers to the questions we are asking.

Cheaper Sequencing, More Genomes

On the heels of the Human Genome Project and the availability of yeast, worm, and fly genome sequences, fly researchers launched new projects with loftier but nonetheless achievable goals, such as a successful effort to sequence the genomes of additional species in the *Drosophila* genus to facilitate evolutionary studies (Drosophila 12 Genomes et al. 2007). As next-generation sequencing technologies continued to improve and the cost of DNA sequencing plummeted during the 2000s and 2010s, even larger projects could be undertaken. Full-genome sequence is now available for many *D. melanogaster* strains, including wild strains captured in a North Carolina farmer's market (Mackay et al. 2012) and from at least five continents (Lack et al. 2015; Lack, Lange, et al. 2016). The genomes of several disease vectors have now also been sequenced, despite the fact that some of these, such as tsetse fly (*Glossina morsitans*), presented particularly challenging cases (International Glossina Genome 2014). Full-genome sequences of the human genome, the *Anopheles* mosquito, and the malaria-causing *Plasmo-*

dium represented the first instance in which the host, vector, and pathogen associated with an insect-borne disease were each sequenced (Beckage 2007). Closing another species-interaction loop, the first genome sequence of a plant that eats flies, the carnivorous bladderwort (*Utricularia gibba*), was published in 2013 (Ibarra-Laclette et al. 2013).

Large-scale sequencing efforts are being made surrounding the human genome as well. Some of these efforts are focused on coverage of an increasing number of primate species (Rogers and Gibbs 2014) and ancient hominids (Paabo 2015) with the assumption that these data will shed new light on human evolution. Other studies focus on uncovering genetic diversity among contemporary humans, such as can be achieved by sampling from populations from different geographical regions (Sudmant et al. 2015; Mathieson et al. 2015). Sequencing of an increasing number of human genomes for studies related to disease is the largest area of focus for research and resources (Hardy and Singleton 2009), and includes sequencing the genomes of everyone from newborns to centenarians, as well as from persons suffering from a wide variety of diseases. One example is the burgeoning field of cancer genomics, wherein the genome sequences of cancer cells from a patient might be compared with the genome sequence of the patient's own healthy cells or with the genomes of cancer cells from other patients (Tomczak, Czerwinska, and Wiznerowicz 2015). Another example is work by the Psychiatric Genomics Consortium, which focuses on uncovering genetic risk factors for a number of psychiatric disorders, from autism and bipolar disorder to anorexia and post-traumatic stress disorder (Psychiatric Genomics Consortium 2017).

Though the goals of large-scale genomic projects in insects and humans diverge, the approaches converge. In both cases, researchers make the assumption that regions that are the same across species or across examples from diverse geographical regions can help reveal what regions of the genome are under evolutionary pressure to stay the same, suggesting that they are critical to function. In human studies, comparing the genome of cancerous and normal cells from the same patient, or identifying changes in common among a large number patients with the same disease or disorder, can help us develop both specific and broad approaches to therapeutics (Chapter 9). Moreover, in applying comparative genomics approaches, we begin to uncover what sequence elements make a fly a fly, or a human a human, at the species and individual levels. As we learn which genes are new or ancient, which genes have been duplicated like adding a second

wrench to a toolbox, and which sequences help turn genes off or on, we gain a deeper understanding of how genomes evolve over time.

The Gene as Concept and as Physical Entity

Altogether, early research performed using the fruit fly *D. melanogaster* dramatically accelerated our understanding of the mechanisms of inheritance—providing key information that brought an accurate understanding to the fore. The gene, once a concept, became a more tangible entity. We now know that genes are made up of DNA, genes are transcribed into RNA, and some RNAs are translated into proteins, with RNAs and proteins forming the main building blocks and machines of our cells and hence our entire bodies. In life, genetics, and fruit flies, it can seem as if everything, every answer to every question, eventually comes down to the level of genes—when they are turned off, when they are turned on, how much of a gene product is made, which versions, and in what surrounding context. We now know that DNA sequences adjacent to genes, as well as other gene-encoded factors, are essential to orchestrating the incredibly complex set of on-off switches and rheostats that control gene expression in a way that makes it possible for a given cell to grow, divide, change, or simply maintain some appropriate status within the whole.

Because as physical entities, genes are, to put it simply, very small, we can more or less understand genes, see them, navigate them, only in the context of maps and sequence data. DNA sequences, in their most accessible form, are long strings of letters in the four-letter code of DNA, functioning like sentences strung together without spaces or punctuation. Maps, sequences, and annotations bring the submicroscopic to human scale and convert the language of complex biochemical building blocks into an interpretable form. Thanks to the early *Drosophila* research, as well as countless other contributions based on studies with diverse organisms and cell types, we have been able to determine that chromosomes are the land along which genes can be mapped, and make visible and navigable DNA landscapes that were previously a mystery. And thus, we can set out—well equipped and rationally guided—to further explore. With an understanding in hand, there was one thing missing: a set of tools with which to better manipulate this newly mapped landscape.

CHAPTER TWO

CHANGE

IN THE EARLY YEARS OF *DROSOPHILA* GENETIC STUDIES, RESEARCHERS worked with mutant strains isolated more or less by chance, with exceptional flies, like the *white* mutant fly, happening to be present in samples collected from the wild or arising spontaneously in a lab culture. Hard work could pay off. The more flies a researcher observed, the better the chance he or she would come across a fly with a visible mutant phenotype. Ultimately, however, finding new mutant flies came down to chance. In 1911, Morgan described a time when flies with mutations affecting the wing "appeared in such rapid succession that my time has been almost entirely consumed in producing pure strains of the new forms" (Kohler 1994). But these so-called epidemics were rare and might be comprised of only four new mutant alleles of one gene over a period of six months. This is progress at a snail's pace by contemporary standards. With current technologies, a single postdoctoral research fellow with access to modest resources could, in less than a year, generate the same number and variety of mutant fly strains that took all the early *Drosophila* researchers working in parallel a total of ten or fifteen years to amass. H. J. Muller, who in 1927 would catalyze a change as revolutionary to biology as the introduction of agriculture was to a previously hunter-gatherer society, summed up the problem this way: researchers, he stated, were "hampered by the extreme infrequency"

of isolating new mutant flies (Muller 1927). It was a problem that needed to be solved.

Due to the "wide-spread desire on the part of biologists to gain some measure of control over the hereditary changes within genes" (Muller 1927), many had attempted to identify what we would now call a "mutagen," some outside agent capable of increasing the frequency at which new mutant strains appear. Researchers tried treating flies and other creatures with a variety of chemicals and other treatments in an effort to find something that would consistently lead to higher rates of mutation, as evidenced by an increase in the frequency of isolating heritable mutations in the offspring of flies exposed to the mutagen. Muller, who had been a PhD student with Morgan, had hints that exposure to X-rays might present a promising approach. In 1923, researchers had reported isolation of two heritable mutations following exposure of mice to X-rays—a promising finding, but numbers too small to be convincing. In 1925, Muller himself published a report detailing the effects of X-rays on recombination in *Drosophila* (Muller 1925). Nevertheless, what if any outside agent could reliably increase the rate of mutation was "highly disputatious at best" (Muller 1927) and full of contradictory evidence.

X-rays as Mutagens

In July 1927, Muller published a study that put the controversy to rest and made him the first to report in the literature definitive proof of the efficient mutation-inducing or "mutagenic" activity of a specific agent, namely X-rays (Muller 1927). In a study conducted over the course of a year, Muller showed that treatment with X-rays of male flies (and hence of their sperm) increases the frequency of uncovering new heritable mutations. It was no accident that *Drosophila* was the species in which Muller was able to definitively demonstrate that X-rays are mutagenic. In the paper, Muller states that he undertook "a series of experiments . . . during the past year on the fruit fly, *Drosophila melanogaster,* in an attempt to provide critical data. The well-known favorableness of this species for genetic study, and the special methods evolved . . . have finally made possible the finding of some decisive effects, consequent upon the application of X-rays," which are "truly mutational" (Muller 1927). Muller was able to state further that "more than a hundred of the mutant genes have been followed through three, four or more generations. They are (nearly all of them, at any rate) stable in their

inheritance" (Muller 1927). Again, *D. melanogaster* proved to be the just-right platform for discovery: the fly's small size, short life cycle, large number of offspring, and easily identifiable visible traits combined to offer up a perfect system for a set of definitive experiments that, like Morgan's work with *white,* sampled thousands of individual organisms and yet took only a year to perform.

In the concluding statements of the paper, Muller discussed the impact his findings might have, not only for *Drosophila* research but also for research in other species. He suggested that "If, as seems likely on general considerations, the effect is common to most organisms, it should be possible to produce . . . enough mutations to furnish respectable genetic maps" (Muller 1927). This turned out to be a gross understatement of the impact mutagens would have on the field of genetics specifically and the field of biology more generally. Mutagens like X-rays—which we now know can lead to deletions, insertions, substitutions, and inversions of the DNA—not only facilitated the drawing of increasingly detailed genetic maps for *Drosophila* and other species, but also provided an essential tool for interrogating the functions of genes. Speaking to the lasting impact of the work, Muller was awarded the Nobel Prize in Physiology or Medicine in 1946. If the Nobel Prize awarded to Morgan in recognition of his contribution to understanding the mechanisms of inheritance is considered the first Nobel Prize for *Drosophila,* then the prize awarded to Muller can be considered the second (with more to come; see Chapters 3, 7 and 8).

Radiation Biology

Muller's work with X-rays helped to spawn an entire field of "radiation biology," which has as its goal exploration of the biological impacts of exposure to high-energy particles, including X-rays, gamma rays, and ultraviolet light. Muller himself was interested in the impact of X-rays on humans, for example, and later studies by *Drosophila* biologist E. B. Lewis and others contributed to an understanding of the relationship between exposure to radiation and cancer, work that had political impact during the Cold War (Lipshitz 2005). An additional finding reported by Muller that turned out to have far-reaching impact in a very different area was that, at high doses, exposure to X-rays could render *Drosophila* sterile. In an adult male fly exposed to a high dose of X-ray irradiation, the DNA of the sperm becomes so damaged that eggs fertilized by those sperm do not develop. Specifically,

this is due to the presence of dominant-acting lethal mutations—evidence of which was provided for the first time in Muller's 1927 report (Muller 1927).

In the 1950s, F. Knipling exploited this ability of X-rays to render insects sterile in the control of the screwworm fly, a nasty parasite that feeds in its larval form on live flesh, making it a significant threat to livestock (Perkins 1977). Knipling persevered over a period of years in designing an effective program to eradicate screwworm that involved release of large numbers of male screwworm flies that had been sterilized by exposure to X-rays. After their release in an affected area, X-ray–treated males mate with wild screwworm females, rendering the resulting fertilized eggs inviable. Application of this "sterile insect technique" was credited in the 1970s with elimination of the screwworm from the southern United States (Perkins 1977). As stated by A. S. Robinson in 2002, the use of radiation-induced sterility to control insect populations "is now a major component of many large and successful programs for pest suppression and eradication" (Robinson 2002). When a new infestation of screwworm threatened native deer in the Florida Keys in the fall of 2016, release of sterile screwworm flies was again used to effectively control the invasion. By March 2017, the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture announced that, following the release of more than 150 million sterile flies, screwworm had once again been successfully eradicated from the region.

Contemporary research in *Drosophila* also connects the field of radiation biology with medicine. In 2012, T. Su and colleagues reported identification of small molecules (chemicals) that enhance the lethal effects of exposing *Drosophila* to irradiation. When fed the small molecule following exposure to irradiation, flies exposed to the small molecule die more quickly or from lower doses of exposure compared with untreated flies. This is bad news for the unfortunate flies. But it might be good news for cancer patients undergoing radiation therapy. Su and colleagues see a similar, enhancing effect of the small molecule on cell death following irradiation of cancer cells, suggesting that what we are learning first in flies might eventually allow researchers to develop drugs that benefit patients undergoing radiation treatment by enhancing the cancer-killing effects of the treatment (Gladstone et al. 2012; Stickel et al. 2015). Starting a route to drug development with fruit fly research might seem like choosing the long road. It surely is. But the long road is not necessarily the wrong road to

take. Particularly if it eventually gets us somewhere we find ourselves grateful to be.

From Random Mutations to Exquisite Control

The finding that X-rays can act as mutagens heralded a revolution in genetics with impact far beyond the study of radiation biology. No longer at the mercy of chance, researchers could now induce mutations at a high rate, opening the door to genetic experiments that built upon past success. Though effective, however, X-rays are not a perfect tool. In a 1928 report describing results that further supported Muller's findings, F. B. Hanson wrote that the researcher using X-rays to induce mutations "may be likened to a hunter shooting birdshot into a flock of ducks . . . it is impossible to aim at any particular gene" (Hanson 1928). Moreover, because X-rays tend to make double-strand breaks in the DNA, they resulted in dramatic, sometimes complex changes to DNA, including deletion or inversion of an entire region of a chromosome. As a result, researchers embarked over a period of twenty years following Muller's work with X-rays to identify chemicals that could act as mutagens, with the idea that chemical treatment might result in subtler changes than were brought about by exposure to irradiation (Auerbach, Robson, and Carr 1947).

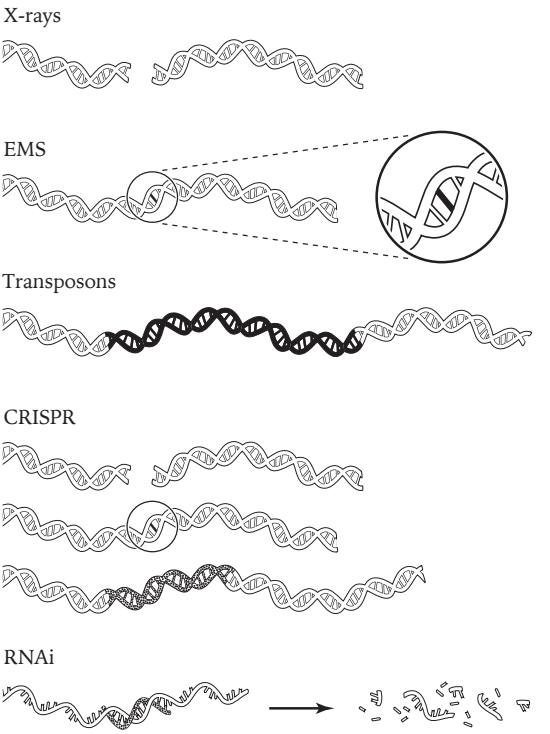
Whereas selection of chemicals tested by others was "mainly random," C. Auerbach applied a reasoned approach, choosing to test the effects of mustard gas, which was known to cause damage to cells that was similar to that generated by exposure to X-rays, such as radiation "burns" on the skin that had difficulty healing. Using *Drosophila* as her test subject, Auerbach succeeded where others had failed. The results of her first experiments treating flies with aerosolized mustard gas, performed in April 1941, were, in Auerbach's words, "spectacular beyond expectation." She observed a dramatic change in the rate of mutation. "Similar results," she stated, "had previously been obtained only with X-rays or other high-energy radiation" (Auerbach, Robson, and Carr 1947). The results had important implications in society at the time and for researchers, and showed that some chemicals are indeed mutagenic, spurring further investigation. An advance for the *Drosophila* research field came when the chemical mutagen ethyl methanesulfonate, or EMS, was introduced into use for fly research in the 1960s. EMS had a distinct practical advantage: it can be added to a sugar water solution and simply fed to flies (Lewis and Bacher 1968; Alderson

1965), making it a relatively safe and easy way for virtually any fly lab to mutagenize large numbers of flies. As it was effective as well as convenient, EMS mutagenesis became a method-of-choice in the *Drosophila* research field and remained so for decades. Mutations induced using X-rays account for about 10,000 mutations cataloged in the FlyBase database; EMS accounts for about 20,000.

In the 1980s, another innovation came along. Researchers developed ways to exploit and engineer "transposable elements," which are naturally occurring DNA fragments, originally identified by B. McClintock in maize, that can "mobilize" or jump around in the genome. In *Drosophila,* transposable elements can be used in several ways to disrupt genes (Venken and Bellen 2012). One way is to induce mobilization, then identify fly strains in which transposable elements are newly inserted into genes, a method pioneered by L. Cooley and A. Spradling (Cooley, Kelley, and Spradling 1988). Transposable elements known to be inserted in or nearby a gene of interest can be mobilized toward another purpose: for some elements, mobilization of the transposon can leave behind a deletion in the original region of insertion, such that mobilization of an element inserted in a region of interest can be used to generate a deletion in that region.

In 1982, G. M. Rubin and A. Spradling reported that transposable elements can also be used as a mechanism by which to introduce genes or other DNA segments into flies (Rubin and Spradling 1982). Specifically, Rubin and Spradling showed that they could restore function of the eye-color gene *rosy* by using a transposon to insert the wildtype *rosy* DNA sequence back into the genome of a *rosy* mutant fly. The approach worked, even though insertion of the *rosy* sequence-containing transposon was in a random location, not in the *rosy* gene, and was done using a relatively short piece of DNA. The demonstration that *rosy* function could be restored by providing a wildtype copy somewhere else in the genome was considered a "critical leap" in the field of gene therapy (Holladay et al. 2012). For *Drosophila* research specifically, the advance opened the doors for insertion of any gene or other sequence into flies for experimental studies, allowing us to do any number of molecular genetic tricks: we can ask whether a human gene can replace the function of a fly gene, introduce a sequence normally found in yeast or bacteria, or make a subset of fly cells glow bright green or cherry red.

Especially after the full-genome sequence of *Drosophila* became available in 2000, researchers were interested in reliable, effective methods that



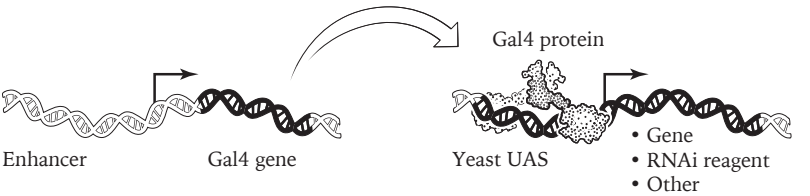
Mutagens used in *Drosophila* research. X-rays make double-strand breaks at random locations, resulting in deletions, inversions, and rearrangements. EMS can have more subtle effects, resulting in changes to single base-pairs of DNA. Transposons cause mutations when they insert into a gene or leave a deletion behind when they exit. CRISPR allows for production of double-strand breaks at specific regions. As such, CRISPR can be used to make small deletions or to insert new DNA. Rather than make DNA changes, an RNAi reagent binds a specific RNA sequence and targets the RNA molecule for degradation, preventing the RNA from being used as a template to make a protein.

not only were efficient at generating mutations but could be customized to target specific genes. Luckily, appropriate technologies came along. In the late 1990s, *Drosophila* researchers began harnessing an endogenous activity called RNA interference or RNAi (first identified in *C. elegans*) that reduces gene function by degrading the RNA rather than attacking the gene itself (Tuschl et al. 1999). Together with the fact that the RNAi reagents can be introduced into flies as artificial genes, making them heritable (Kennerdell and Carthew 2000), the end effect is similar to the effects of a

mutation, but with the significant advantage that we can design an RNAi reagent to target a specific gene—an approach known as "reverse genetics" since the gene sequence and not random mutagenesis serves as the starting point of the study. A next wave of power over the fly genome came with the development of CRISPR-based technologies for gene disruption, gene engineering, and other perturbations. Like X-rays, CRISPR technology can be used to make double-stranded breaks in DNA. Unlike X-rays, however, with CRISPR the double-strand breaks can be induced with precision, targeting a predefined location within a gene or in another region. To extend Hanson's duck hunting metaphor, with CRISPR, we can do the equivalent of shooting a specific feather on a specific duck, or replace that feather, or the whole duck, with another of our own design.

The Experimental On-Off Switch

Adding further power to genetic approaches available for studies with *Drosophila* was the development by A. Brand and N. Perrimon of the Gal4-UAS system, a method of gene activation that borrowed its component parts from yeast (Brand and Perrimon 1993). At its simplest, the system is made up of two parts, a protein called Gal4 and a specific DNA sequence that is bound by the Gal4 protein, the "upstream activation sequence" or UAS. Brand and Perrimon exploited the property that when the two components are brought together in a fly—that is, when Gal4 protein is produced and binds the UAS—the normal transcriptional machinery of the fly is recruited to the UAS site, such that any cell in which Gal4 protein is being made produces a lot of RNA from whatever sequence is next to the Gal4-bound UAS sequence. Either through insertion of Gal4 in the genome, which in some cases will place it near a fly genome enhancer that will cause Gal4 to be expressed in a specific subset of cells, or by engineering a DNA sequence in which a fragment of an enhancer controls Gal4 expression and introducing this into the fly, we can identify flies in which Gal4 is expressed in all cells and during all stages of development, in some cells or at some life-cycle stages, in very few cells or one stage, and so on. These Gal4-expressed fly stocks, then, can be combined with flies in which UAS is placed upstream of a gene, RNAi reagent, or other sequence. That sequence will be transcribed or turned on only in those cells and life-cycle stages in which Gal4 is produced.



The Gal4-UAS system: an on-off switch for genetic experiments. The Gal4 gene can be introduced into flies in random or defined regions, such that Gal4 expression comes under the control of a fly "enhancer" region that induces expression of Gal4 in specific cells, tissues, or organs. In cells in which it is expressed, Gal4 protein will bind to the upstream activation sequence (UAS) and turn on transcription of a nearby sequence, which could be a fly gene, an RNAi reagent sequence, or another engineered sequence.

When the sequence controlled by the Gal4-UAS system encodes an RNAi reagent, in any cell expressing Gal4, the RNAi reagent is made and the gene target is "knocked down" or reduced in RNA levels, which typically results in a corresponding reduction in the levels of the protein encoded by the gene. Targeting the *yellow* gene with RNAi in the fly wing, for example, results in yellow pigmentation phenotype in the wing, whereas the rest of the fly's cuticle will be normal in color. The Gal4-UAS system can also be combined with CRISPR technologies, allowing researchers to generate mutations only in cells that express Gal4 (Port et al. 2014; Port and Bullock 2016), or boost expression of specific genes (Lin et al. 2015), again in a tissue- and life-cycle stage-specific manner. When combined with RNAi or CRISPR, the Gal4-UAS system makes it possible to preserve normal function of a gene in some life-cycle stages and cell types, and disrupt gene function only in the stage or cell type under study. In this way, we can ask what happens when a gene is disrupted in adults even when the gene is normally required in embryos for development, or when a gene is disrupted in the eye even when the gene is normally also required in the wing and other structures.

The Gal4-UAS approach has gained additional power over time. Related strategies and permutations of the original system further refine the ability to turn genes off and on. It is now possible to use temperature to turn Gal4 off or on, for example, so that a gene can be activated or inactivated for a period of just a few days. In addition, the number of fly stocks in which Gal4 is expressed in patterns defined by different fly enhancer sequences

has expanded dramatically, a result of the combined efforts of individual labs and larger-scale efforts. The Bloomington *Drosophila* Stock Center maintains and distributes more than 5,000 Gal4 fly stocks, with Gal4 expressed in a different subset of cells in each one. In 2012, A. Jenett, G. M. Rubin, and colleagues generated a systematic collection of tens of thousands of Gal4 fly stocks that facilitate, among other things, expression of genes or other sequences in specific sub-regions of the brain (Jenett et al. 2012). Altogether, fly stocks that facilitate use of the Gal4-UAS approach provide a unique and precise toolkit for experimentation.

Finding Balance

Another critical tool in the *Drosophila* biologist's toolkit grew from Muller's work with X-rays: the so-called "balancer" chromosomes. Muller was able to isolate, following irradiation, fly stocks with multiple large rearrangements in the order of genes along their chromosomes. Imagine a giant picking up a section of a city street, complete with its bordering buildings, then turning it 180 degrees and plopping it back down. All the buildings would be there, lining the street. But the order in which they appeared relative to other sections of the street would be inverted. Likewise, when DNA is broken in more than one place, it can be pasted together again by the cell in an opposite direction. One of the effects of inversions on the chromosome sequence is to make it difficult for partner chromosomes to line up, preventing those regions from undergoing crossing-over and recombination (Muller 1928). Balancer chromosomes were also selected to contain at least one recessive lethal mutation, so that flies homozygous for the balancer chromosome do not "take over" the fly stock after several generations, and to have at least one dominant mutation with an easily identified phenotype (such as curly wings), so flies with the balancer chromosome are easy to spot. Thus, when one copy of a fly's chromosomes carries, say, an intriguing mutation, and the other copy of the chromosome is one of these multiply inverted "balancer" chromosomes, the mutation stays where it is, and we can identify it as the chromosome that does not carry the dominant visible mutation.

Combined, these properties make it possible to faithfully preserve, within a continuous lineage, flies that carry lethal or otherwise deleterious mutations that would normally be lost over time. Generation after generation, the only offspring that survive will be those with one copy of the lethal

mutation of interest and one copy of the balancer chromosome, since flies homozygous for either would be dead. Many mutations that would otherwise have been lost, including mutations that cause lethality, sterility, or other deleterious effects, have been preserved, like rare books in a special collections room, through the continuous culture of the mutations in the form of "balanced" fly stocks.

Drosophila as Mutagen Detector

Both Muller and Auerbach used *Drosophila* as a test system in which they were able to demonstrate that a treatment or chemical was mutagenic. The use of *Drosophila* as an indicator species of this kind has been exploited ever since, including to assess the ability of other treatments to induce mutations and determine the efficiency at which they do so (Kilbey et al. 1981). In 1984, U. Graf and colleagues reported a particularly sensitive method for the detection of mutagenic activity (Graf et al. 1984). Although originally called the "somatic mutation and recombination test" or SMART, the approach became known as the "wing-spot test" because the test uses the fly wing—a conveniently external tissue—as a living Petri dish in which the presence or absence of a specific visible mutation is used to measure the frequency of "somatic recombination," a chromosomal event indicative of breaks in the DNA. The wing spot test relies on the availability of a recessive mutation that affects the fine hairs present on a fly's wings, such as a mutation in the gene *multiple wing hairs* (*mwh*), which results in a forked cluster of hairs in places where in wildtype, only one wing hair would form.

Normally, the type of crossing over associated with linkage analysis occurs only during formation of the germline cells, the cells that form the sperm or eggs. When DNA is damaged, however, that type of crossing over can occur in somatic cells—that is, cells other than germline cells. A fly that is heterozygous for a mutation in *mwh* would normally have wing hairs indistinguishable from wildtype. If during development the fly is exposed to a mutagen that causes DNA damage, then somatic recombination can occur during the normal division of cells. This can result in the formation of a "clone" or population of cells, each derived from the same progenitor, that inherit two copies of the *mwh* mutant chromosome and no wildtype copy. In this patch of cells, the wing hairs will be forked, a phenotype that is easily observed using a student-grade microscope. A mildly mutagenic agent will result in a small rate of somatic recombination, producing just a

few patches of cells with the *mwh* mutant phenotype. By contrast, a potent agent will result in a higher rate of somatic recombination, thus producing more patches or "spots" on the wing in which there are forked wing hairs.

The wing-spot test allows researchers to reliably and quantitatively assay the mutagenic activity of poisons, other chemicals, or treatments in a whole-animal system (Graf et al. 1984). Indeed, at least as late as 2013, *Drosophila* was the only available in vivo system for detection of somatic recombination, an event associated with mutagens and with cancer (Marcos and Carmona 2013). The wing-spot test has been used to measure the mutagenic activity of hundreds of different compounds, including known poisons, therapeutic drugs, plant toxins, fungal toxins, magnetic fields, and more recently, quantum dots and nanoparticles (Vogel et al. 1999; Koana et al. 1997; Alaraby, Demir, et al. 2015; Chifiriuc et al. 2016; Carmona et al. 2015; Alaraby, Annangi, et al. 2015). The test can also be used to identify natural products or chemicals that lower the rate of mutagenesis; in this case, the test is used to ask whether co-treatment with some chemical of interest and a known mutagen offers some level of protection against the damaging effects of the mutagen, reducing the number of mutant clones as compared with a control group that receives the mutagen but is not co-treated with the potentially protective compound (Graf et al. 1998).

The Power of the "Genetic Approach"

With X-ray and chemical mutagens available to speed the pace at which researchers could isolate new mutant strains, the field of genetics largely shifted during the early twentieth century from studying the mechanisms of inheritance to using this new understanding of chromosomes, genes, and mutagenesis as a tool for studying other areas of biology. At the crux of these studies is the "genetic approach" to uncovering gene function. The genetic approach rests on the idea that if we reduce or remove the function of a gene through mutation, and then observe the consequences of that reduction or removal of gene activity on the cell or organism, we can identify what function is missing. In this way, we can come up with a reasonable hypothesis regarding what the normal function of the gene might be. Remove the starter motor from a car and it fails to start; ergo, the starter motor is likely to be involved in the process of starting the car. Remove the tires, the steering wheel, or the dome light, and the consequences are quite different;

these parts are involved in different sets of functions. To learn about a given cellular process or activity of the organism, we simply change what mutant phenotype or defect we look for. Continuing the idea of biological system as a car, whereas one group might be interested in what parts are required for the car to start or the engine to run, another might be more interested to learn what set make for a comfortable backseat or are required to clear the fog from the windshield.

Researchers tailor their "assays" or tests—what mutant phenotypes they are looking for—according to the process they are interested in studying. A general truism of the genetic approach is that the more specific the question we ask, the smaller the number of parts (genes) we expect to be involved. The repair shop equipped with a lift and precise digital sensors can tell us more specific information about what's wrong with a malfunctioning motor and more readily pinpoint the small number of parts that need to be replaced. Another way to think of the genetic approach is as a system by which we can group genes into functional categories. The more specific the assay or test, the more granular and specific the categories, with groups of genes further divided into subgroups as mutant strains are run through an increasingly specific battery of tests.

The application of the genetic approach at large scale is called a "forward genetic screen." The idea behind a screen is simple. First, choose a mutant phenotype that is indicative of what might go wrong when the process, structure, or behavior of interest is disrupted. This is the "screen assay." Next, create or obtain a set of mutant flies, such as by mutagenizing them with EMS, and then ask which subset of the mutagenized offspring "score positive" in the assay, displaying the mutant phenotype of interest. The limits of what can be tested are bounded only by the topic under study, the research team's creativity, the availability of technologies that made it feasible to perform the assay on a large number of flies, and practical considerations, such as the time and money it would take to perform the assay on a large number of flies. A selected list of genetic screens performed in *Drosophila,* representing different screen strategies, mutagens, and screen assays, is presented in Appendix C, and an example of a forward genetic screen with particular impact is presented in Chapter 3.

Disruption of most genes will not affect the outcome of the assay, whereas an interesting subset of mutagenized flies will score as positive; these flies are collected and propagated for further study. Performing a screen is often an endurance event. In a typical screen for homozygous recessive mutations

that result in a given phenotype, researchers must mutagenize a large number of male flies of a convenient genotype; set up individual paired matings between the mutagenized flies and some other genotype (often adding up to tens of thousands of paired matings, each in a separate food vial; this is termed the parental or P_0 cross); set up at least one subsequent cross among the progeny of the first cross to obtain homozygous mutant animals (the F_1 cross); then perform the screen assay on the flies in the next generation (F_2) that are of the right genotype, in order to identify the small subset that display the mutant phenotype of interest.

Why expend the effort required, which involves "pushing" tens or hundreds of thousands of flies and assaying the resulting offspring, most of which will not have the mutant phenotype of interest? The answer lies in the outcome—in the few fly strains that score as positive in the screen assay. The forward genetic screen is to the genetic researcher what the site dig is to the archeologist or fossil hunter. If the location is well chosen and the dig is planned and executed carefully enough, then there is a reasonable chance that new information will be gathered, new specimens unearthed. Exactly what will be found remains a mystery until the dirt is cleared, but success is likely. Similarly, a genetic screen allows researchers to identify, with no imposed bias as to which genes we think might be involved, the sets of genes that have in common that they score as positive in the assay. If we think of a genome sequence as providing us with a parts list, then we can think of the application of the genetic approach, including in large-scale genetic screens, as helping to "assign function" to those parts—to understand what each of those parts actually does.

The Impact of Understanding

In 1949, H. J. Muller published a statement in the inaugural issue of *American Journal of Human Genetics,* a journal he helped found, that included a couple of prescient predictions. Foretelling the power of model organism research to uncover findings that apply to our understanding of ourselves, Muller stated that "much of this genetic dissection of development and physiology [of humans] can be carried out most profitably through pilot work on laboratory forms." He considered it "likely that many results" from vertebrate animal studies "will later be found to be represented by homologous human cases." This has proved true not only for vertebrates as Muller predicted, but also for *Drosophila* and other invertebrates. In the

statement, Muller went on to make another prediction that has new relevance in the current post-genomic era. "On the other hand," he stated, "men are examined intensively in so much greater numbers than any animal, that the human material itself will often present the pilot findings, which can later be followed out in more controlled fashion in animals" (Muller 1949).

This "intensive examination" of humankind now includes sequencing the genomes of an increasing proportion of the populace. As a result, we are at a critical time with regard to the diagnosis and treatment of human genetic diseases, many of which have their impact on children, and with regard to our understanding of the genetic contributions to diseases that are influenced by genes but not strictly inherited, including various cancers, neurodegenerative diseases, diabetes, obesity, and inflammatory diseases. With next-generation sequencing as a tool, and an increasing number of people serving as the study set, experts in medical genetics are trying hard to link diseases to their inherent genetic causes or contributions, and identify new genes relevant to susceptibility or resistance to disease. Today's technologies make it relatively easy to detect "variants" or differences in the DNA sequence of protein-coding regions of genes or other regions of the human chromosomes. One problem is that finding variants is, in a sense, too easy. Take any person's genome and we will find variants—hundreds or more—compared to some reference human genome or even the genome sequence of a parent or sibling. We are, after all, each unique. Another problem is that for the genes identified in this way, we often do not have sufficient information regarding the function of the gene, hampering efforts to use the gene information to develop a therapeutic strategy.

As a result, a key challenge in using genome sequence data in diagnosis and treatment of individual patients, or to identify genetic risk factors of disease, is to figure out which of the many differences detected in an individual's genome, or in population-based studies, have a relevant impact on health, and how the gene's function is relevant to disease. *Drosophila* research contributes to these efforts in at least two significant ways. First, researchers are using *Drosophila* as a platform to sort the true disease-causing differences in human genes from the red herrings (Ramoni et al. 2017; Bellen and Yamamoto 2015) (see Chapter 9). Second, the vast body of knowledge already gained through fundamental studies with *Drosophila,* as well as the new knowledge we uncover every day in new studies, helps inform our understanding of human gene functions, giving us answers to the "how"

question of disease association. Studies in flies—some of them using mutations isolated in the early years—have contributed to our ability to predict which genes are related to a given disease, to build meaningful hypotheses regarding how the genes normally function and how disruption of those functions leads to disease, and to design effective therapeutic drugs and treatments.

For *Drosophila* and other models, this work of assigning gene function—learning what the genes do—inevitably cycles back to the ability to efficiently generate mutations, as first reported by Muller, and to study the resulting phenotypic outcomes. No approach has done more to inform our understanding of gene function than the genetic approach, including its use in forward genetic screens. As stated by J. L. Rinn in 2013, "Genetic approaches are as fundamental to biology as math is to physics" (Doolittle et al. 2013).

CHAPTER THREE

COMMUNICATION

THE CELLS IN A MULTICELLULAR ORGANISM ARE MEMBERS OF A COMMUnity, and like any community, its members have a need to communicate with one another: to provide status updates, ask for directions, even get into the occasional argument. Communication among cells is particularly important during development, the process by which a single fertilized egg becomes an adult. A developing embryo, be it fly, frog, human, or other, does not simply undergo cell division and growth. It also forms distinct cell types, each with a defined identity and associated with a set of specialized activities appropriate to its position within the whole. Doing this right—with all the right cell types forming or ending up in all the right places—depends upon communication among cells. But in addition to a need to communicate, cells have an obvious barrier to doing so, in the form of the plasma membrane, an oily skin that surrounds each cell and defines the difference between what is "extracellular" or outside the cell and what is "intracellular" or within it, which includes the cytoplasm, nucleus, and other organelles. For a cell to receive, interpret, and respond to a piece of information, that information must somehow be communicated across the plasma membrane. Some molecules can pass through the membrane; most proteins and large molecules cannot. Thus, sending protein or chemical messages from one cell to the next requires not only sending a

signal, with the right cells sending the right messages at the right times, but also a process called "signal transduction," in which information received at the cell surface—outside the plasmid membrane—is transduced or relayed within the cell itself, eliciting a response.

In considering the impact of new findings in the area of cell communication, we can ask a number of questions. What happens if the signals that go right in development—inducing cell growth, division, movement—are sent at the wrong times or to the wrong tissues? If signals are scrambled, miscommunicated, or ignored? Like any breakdown of communication, such a disruption can wreak havoc, leading to chaos, misdirection, and general discord. Cells lose the identities they took on during development. They stop responding to new information or respond in inappropriate ways, and might send new messages they should not. A key outcome of such a breakdown of cell-to-cell communication and signal transduction within cells, and the loss of cell identity such a breakdown brings on, is inappropriate growth and proliferation of the cells. Without signals that tell them to stop, cells keep growing and dividing, either within the tissue in which they originated or, in some cases, after moving to other locations.

If this sounds familiar, it should. We know this outcome—inappropriate growth and proliferation of cells—by another term: cancer. Thus, by learning the mechanisms by which cells communicate during development, we learn, too, about genes and cell activities relevant to cancer, and how their disruption devolves into disease. Much of the knowledge we have gained about communication has its roots in *Drosophila* research, and indeed, one genetic screen—and the work that followed—opened a path that connects the full intellectual distance from fly-pushing station to clinical application.

Defining Order

The hatching of a *Drosophila* larva from the eggshell is a major rite of passage. To reach this stage, a single cell—the fertilized egg—must divide, reorganize, retool, and form specialized tissues, with only the contents of the egg and its newly formed diploid genome (half from mom, half from dad) to get things started. One of the questions of interest to developmental biologists has been how a multicellular organism sets up the major body axes, such that the head ends up at the head end, the rear at the rear, and everything properly organized in between. Various lines of evidence have

contributed to our understanding of how this occurs. For example, based on the outcomes following transplantation of tissues (such as those of a frog) from one developing embryo into another, researchers had become convinced that a variety of activities were present in embryos, including the activity of what became known as "morphogens," specialized molecules that diffuse across the embryo, sending a message that helps define the anterior-posterior (front-to-back) or dorsal-ventral (back-to-belly) axis. The assumption was that high levels of a morphogen instruct cells to make one set of structures, whereas moderate levels instruct cells to form the middle forms and low levels or the absence of the morphogen direct the formation of structures that should be present at the opposite end.

Proposing the existence of morphogens and other activities is one thing; identifying what gene or genes encode those activities and how they work at the molecular level—how a gradient of morphogen activity forms, how a morphogen communicates information to cells, how that information is transduced and interpreted, bringing about the right outcome—is another thing altogether. In many cases, the answers to how a process occurs came only after researchers began to take a genetic approach, including the use of forward genetic screens. But, of course, before the genetic approach can be applied as a tool for the study of a topic, researchers need an assay, a test, a mutant phenotype to look for. Something that would indicate that the processes of interest had been disrupted. For the study of developmental biology, researchers might seek an indication that a morphogen was absent or altered, or that the message the morphogen was meant to send was not received.

The Larval Cuticle Phenotype

The larvae of some Dipterans hatch from their eggshells with snorkel-like extensions, as is common among aquatic Dipteran larvae, or darkly pigmented bristles, as is true for the bot fly. By comparison, *Drosophila* larvae are stripped-down, generic models, soup cans without labels. Nevertheless, they have a few modest but distinguishing features that help orient researchers along the anterior-posterior and dorsal-ventral axes. For example, even after a researcher has used chemicals to strip away all the soft bits of the larva, leaving only the tough outer cuticle, like a shed snakeskin, it is easy to recognize the extreme ends, such as by observing the mouth parts and anal pads. In addition, along the anterior-posterior

axis of the cuticle, we can observe distinct "segments" or subregions, classified as three thoracic segments (T1-T3) and eight abdominal segments (A1-A8). There are also differences along the dorsal-ventral (back-to-belly) axis. On the ventral side of the larval cuticle, each segment has visible "belts" or stripes of fine hairlike structures called denticles; on the corresponding dorsal side, the cuticle is "naked" (clear, featureless, and smooth). The denticle belts, along with the head and tail structures, define the major axes of a larva's body, and allow researchers to distinguish countable units—the segments—which differ just enough with regard to the size and shape of the denticle belts present that each segment can be distinguished from the others.

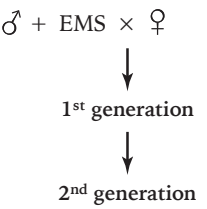
With these axes of a larva serving as guideposts, *Drosophila* researchers were able to look for exceptions. Mutations that disrupt formation of the major body axes can be recognized based on disruptions of the larval cuticle pattern—such as missing or duplicated segments. One of the first such mutations isolated and characterized was *bicaudal,* in which the cuticles of mutant larvae have two back ends and no front (Nüsslein-Volhard 1977). Since in *bicaudal* mutant cuticles, the more anterior segments are missing, applying the genetic approach we can conclude a normal role of the *bicaudal* gene product in specifying anterior structures of the developing larva. But *bicaudal* is just one gene. Nothing could be more fundamental to a multicellular living creature than organizing the embryo into its various regions, and making the right cells in those regions. Surely there must be other genes—whole sets of interrelated gene networks—required to make something like a larva, with its axes defined, its body regions, segments, and other structures in place, attributes that will later be echoed in design when the larva forms a pupa and then an adult. We can ask, how many genes are involved? What are they? And how do they set up body axes and subdomains?

Developmental biologists, who study how a fully formed adult grows from a fertilized egg, were interested in knowing the answers. The hunt for these other genes, as well as the pursuit of some other intriguing phenotypes relevant to the development of an egg into an adult, such as formation of an eye with an unusually smooth or rough texture, turned out to reveal a surprising number of fundamental truths about how a cell can communicate information to another, and, just as important, how the receiving cell internalizes and interprets the message, and responds.

The Most Famous Genetic Screen

Arguably the most famous forward genetic screen is a screen done by C. Nüsslein-Volhard and E. Wieschaus in the late 1970s, in which the researchers screened for mutations that led to disruption of the normal organization of the larval cuticle (Nüsslein-Volhard and Wieschaus 1980). In designing the screen, which became known as the Heidelberg screen (St Johnston 2002; Wieschaus and Nüsslein-Volhard 2016) and led to Nobel-prizewinning findings, the researchers aimed to do in the fly what geneticists working in simpler systems such as yeast or viruses had already done with success. That is, they set out to perform a "saturating" genetic screen, in which an attempt is made to identify not just some but all genes for which disruption of the gene can result in a given mutant phenotype—in this case, mutation of all genes for which disruption of the gene alters the stereotypical pattern of the larval cuticle. Saturating genetic screens performed in less complex organisms had already proved productive, identifying, for example, the complete set of genes required in "bacteriophage" (viruses that infect bacteria) for assembly of the "coat" that encases the virus (Calendar 1970). These screens ultimately allowed researchers not only to build a catalog of bacteriophage genes, but also to establish some of the first "gene networks" describing the functional connections among gene activities, something that might be likened to working out the order in which a set of specialized machines are used to build an end-product from starting materials.

Nüsslein-Volhard and Wieschaus reasoned that, despite the increased complexity of a fly larva as compared with a bacteriophage, they could apply the same approach to defining a list of genes required to build a properly organized larva and eventually work out an ordered pathway of interaction among those genes. The idea of attempting a saturating screen of a much more complex process—establishment of an organized body plan in a multicellular organism—was a bit radical for the time. There were indications, however, that the types of mutant phenotypes the researchers sought were possible to find, such as the existence of *bicaudal* mutant flies (Nüsslein-Volhard 1977; Nüsslein-Volhard and Wieschaus 1980). As Nüsslein-Volhard and Wieschaus began to screen, they soon found that they could identify mutant phenotypes that disrupt the regular pattern of the larval cuticle. One of the first of these identified, *dorsal,* resulted in the complete absence of ventral denticle belts, with the whole cuticle made up of dorsal-type, naked cuticle (Wieschaus and Nüsslein-Volhard 2016). Altogether, by the



The Heidelberg screen strategy. Male flies are treated with EMS and mated to females. First-generation siblings are mated to one another. A quarter of their offspring will be homozygous for a recessive mutation, and some of those mutations will result in defects to the larval body plan, identified by examination of the cuticle.

end of the screen, the researchers found that they could identify several mutations in each of a few categories: "pattern duplication in each segment"; "pattern deletion in alternating segments"; and "deletion of a group of adjacent segments." The researchers termed these segment polarity, pair-rule, and gap mutants, respectively (Nüsslein-Volhard and Wieschaus 1980).

The goal of the Heidelberg screen was to gain insights into the genetic underpinnings of development in this already much-studied fly—to gain a more comprehensive picture of the number of genes and nature of the gene products (RNAs and proteins) required for the profound changes associated with turning a one-celled embryo into something ordered, segmented, and patterned. In their 1980 report of the screen, Nüsslein-Volhard and Wieschaus described the identification in the screen of new mutations in five previously characterized genes, along with mutations that mapped to ten new genes (Nüsslein-Volhard and Wieschaus 1980). The previously identified genes were *cubitus interruptus, wingless, fused, engrailed,* and *Krüppel.* The newly identified genes were *gooseberry* and *paired,* early examples of what became known later as PAX family genes ("PAX" for "paired box"), "orthologs" or relatives of which were later found in the human genome; *even-skipped,* which turned out to have two human orthologs, named after the fly gene *EVX1* and *EVX2*; *odd-skipped,* which has the human orthologs "odd-skipped related" or *OSR1* and *OSR2*; *barrel,* which is related to human HES family genes; *runt,* which defined the "Runx" family, with human orthologs *RUNX1*, *RUNX2*, and *RUNX3*; *knirps* and *hunchback,* neither of which has obvious close relatives in the human genome; and one other, a gene the researchers named *hedgehog* (*hh*).

10,000 Hedgehogs and Counting

A search with the term "hedgehog" in the U.S. National Center for Biological Information's PubMed database of biological and biomedical research papers retrieves more than 10,000 research and review articles, published in a wide variety of scientific journals. With very few exceptions, these articles have no relationship to the animal known as the hedgehog (family Erinaceinae) but trace back to the *hh* mutation isolated in the Heidelberg screen. The gene got its name from the observation that, in *hh* mutant larval cuticles, the denticle belts are present but the intervening stripes of naked cuticle are absent, so that overall, the cuticle is shorter and hairier than a wildtype larval cuticle, and so resembles the spiky animal. The specific disruption observed in *hh* mutant larval cuticles, with the half-segment of naked cuticle missing from each segment, turned out to be a first clue, a first insight into a system of communication that became known as the Hh signal transduction pathway (Hh pathway). The components of the Hh pathway provide one way for cells to send a message or signal (via the Hh protein, which is released by one cell and travels to reach the surface of others), receive a Hh signal at the cell surface, and transduce the signal within the cell. Importantly, with the information conveyed past the barrier formed by the plasma membrane and "transduced" within the cell, the signal can elicit some set of responses, such as changes in cell shape or movement, changes in the set of genes that are turned on and off in the signal-receiving cells, or both.

Building on genetic study of the fly, and with research in other systems contributing to and converging with what had been learned in *Drosophila,* researchers were able to figure out both what the components of the Hh signal transduction pathway are and in what order they act, with the activity of one protein dependent on activation of another. The Hh protein binds to a receptor embedded in the cell surface that in *Drosophila* is encoded by the *patched* (*ptc*) gene. Binding of the Hh ligand to Ptc changes the interaction between Ptc and another membrane-spanning protein, encoded by the gene *smoothened* (*smo*), in turn changing the stability of the product of the *cubitus interruptus* (*ci*) gene. Ci protein is a "transcription factor," a protein that can bind DNA in specific regions, typically just in front of the protein-coding region of a gene, and turn on or off a nearby gene, leading to other changes within the cell. Together, the Hh, Ptc, Smo, and Ci proteins make up the core components of the Hh signal transduction

pathway—they are the buckets in a bucket brigade aimed at conveying information within the cell. The signal is received at the cell surface (receipt of Hh by Ptc), passed along via transduction of that signal inside the cell (an Hh-dependent change in the interaction between Ptc and Smo, which in turn changes the stability of Ci), and elicits a response (in the form of genes turned off or on by the Ci transcription factor).

Eventually researchers realized that the Hh pathway is used as a communication not only during embryogenesis but also at other stages in the fly's life cycle—with the pathway wired up similarly and reutilized in different stages and tissues to help solve similar problems. Hh pathway signaling is required at all stages in the fly life cycle, in embryos, larva, pupae, and adults; for the proper formation of the eyes, wings, antennae, and other structures; in the female and male reproductive organs; in the brain and other nervous tissues; for migrating cells to reach their targets; and more. In addition, as fragments and then complete sequences of genes and genomes became available, researchers realized that the Hh pathway components are reused not only within the fly, solving the same problem in various contexts, but also in evolution, with the pathway solution to communication co-opted to solve new and additional problems. Indeed, Hh pathway components and the way they function together have been conserved in other organisms. Many other animals, including we humans, contain versions of the protein components of the Hh pathway, and furthermore, the wiring up of those components—the order and direction in which they act and the types of interactions they have with one another—are comparable.

The three *hedgehog*-related genes identified in the human genome were shown to similarly act as ligands, and were given names that pay homage to the pioneering *Drosophila* gene: they were named *Sonic hedgehog*, *Indian hedgehog*, and *desert hedgehog* (abbreviated *SHH*, *IHH*, and *DHH*). Like Patched, its human orthologs, PTCH1 and PTCH2 (Johnson et al. 1996; Carpenter et al. 1998), are receptors for the human Hh-related proteins; the human ortholog of Smo, known as SMO (Sublett et al. 1998), interacts with human PTCH proteins; and the human orthologs of Ci, known as GLI1, GLI2, and GLI3 (Ruppert et al. 1990; Tanimura, Dan, and Yoshida 1998; Ruppert et al. 1988), act as transcription factors, turning genes on or off in response to receipt of a Hh pathway signal. Altogether, and despite the wide gulf that separates fly and humans in evolutionary time, as well as the marked difference between putting stripes

on a fly larva and forming very different structures during our own development, the fly and we humans share an ancestor in which the Hh pathway evolved, and cells in both organisms use the Hh pathway as a mode of communication.

Sameness and Difference

How do we learn that a fly gene has one or more equivalents in the human genome? Comparison of the amino acid sequences encoded by genes is both the easiest and the most common method by which to do this. Proteins with similar amino acid sequences often turn out to have the same or related functions at the biochemical and cellular levels. However, amino acid identity and other indicators of orthology relationships are imperfect predictors as to whether or not two similar proteins have the same function. Researchers were excited when they identified a protein in *Drosophila* that has 40 percent amino acid identity compared with the firefly luciferase protein, which is responsible for that insect's flashes in the dark. When they made the fly protein and tested it with the components that for firefly luciferase would result in production of light, however, they saw no evidence of a light-producing function for the fly protein (Oba, Ojika, and Inouye 2004).

Conversely, there are proteins that have more limited similarity at the level of amino acid sequence but are known to have the same function, demonstrated by the finding that one can substitute for the other in a living system. The fly ortholog of the human gene *TP53* (better known as "p53"), for example, has only 23 percent amino acid identity to the human protein but has comparable functions (Jin et al. 2000). In addition to asking whether there are similar proteins shared between flies and humans (in essence asking, do the two languages have similar words?), we can also ask whether the two species have a common ancestral gene (is there a common root word from which each derived?) or whether they are similar in three-dimensional structure. The definitive experimental test for defining a close relationship between a pair of genes or proteins is a kind of swapping, in which we ask whether the gene encoding the human (or other) version of a protein can replace the function of the fly gene, or vice versa.

Conservation of signaling components and their functional relationships to one another has, particularly in the post-genome era, become a recurring theme. Human cells rely on basically the same genes when it comes to

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ASGPLEGVIRRDSPKFKDLVPNYNRDILFRDEEGTGADRLMSKRCKEKLNVLAYSVMNEW
ASGRYEGKIARSSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDRLNSLAISVMNQW
ASGPAEGRVARGSERFRDLVPNYNPDIIFKDEENSGADRLMTERCKERVNALAIAVMNMW
ASGRYEGKISRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQW
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PGIRLLVTESWDEDYHHGQESLHYEGRAVTIATSDRDQSKYGMLARLAVEAGFDWVSYVS
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PGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYES
**:.* ***.**** **.::********: *:*****..***:************* * *

RRHIYCSVKSDSSISSHVHGCFTPESTALLESGVRKPLGELSIGDRVLSMTANGQAVYSE
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KAHIHCSVKAENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSD
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Comparison of fly Hh protein to its human orthologs IHH, DHH, and SHH. Top line, part of the fly Hh protein sequence; next three lines, equivalent subregions of human orthologs. Amino acids are represented using a standard single-letter code. On the last line, an asterisk indicates amino acids that are the same in all four proteins; a colon, chemically similar amino acids; a period, chemically related amino acids.

how cells communicate as flies do, as well as worms, fish, other animals, and even plants and fungi. Moreover, just as we can communicate via different mechanisms, using phones, text messages, and paper notes, so, too, cells have multiple ways to communicate: multiple signal transduction pathways. *Drosophila* research contributed to identification of founding members and additional components of these pathways, leading us toward a "universal view" of how animals develop (Shilo 2016). At least the following pathways are found in both flies and humans, with the core components and their relationships the same or similar in both species: the circadian clock pathway, the Dpp/TGF-beta pathway, the EGFR and PVF pathways, the FGF/FGFR pathway, the Hh pathway (this chapter), the Hippo pathway (Chapter 4), the Imd pathway, the Insulin pathway, the JAK/STAT pathway, the planar cell polarity pathway (Chapter 5), the Notch pathway (Chapter 5), the nuclear hormone receptor pathways, the Slit-Robo pathway (and other cell guidance pathways), the TNF-alpha pathway, the Toll/TLR pathway (Chapter 7), and the Wnt pathway. Many of these were named based on a mutation in a *Drosophila* gene that led to identification of the founding member of the pathway. The Wnt pathway was named for the fly gene *wingless* and the mouse gene *int1*; the Notch pathway was named for the fly gene

Notch; *hh* gave us the Hh pathway; the list goes on. More important, research in the fly has contributed a tremendous amount to our understanding of individual components of signaling pathways, the order of their actions within a pathway, and the types of outcomes they can control. This information provides researchers focused on studies of similar genes in humans (or other species) with reasonable guesses regarding the functions of their orthologs in human cells and organs—guesses that can be tested in a focused and efficient manner, revealing new information about communication among our cells.

Glove and Mitt, Action and Reaction

Signal transduction can be likened to a sport, with players acting together as a team to accomplish some common goal, and with the specific inputs—what pitch the opposing team's pitcher might throw, for example—predictable but not certain, requiring a degree of flexibility in the team's response. A ligand binding to a receptor, inducing some reaction within the cell, is easily compared with a softball player catching a ball in her mitt. Subsequent throws of the ball from player to player parallel the conveyance of information, through molecular modifications, that effect some eventual outcome. Like the roles assigned to players at various positions on the field, the identity and subcellular localization of proteins often define how the proteins respond to receipt of the signal. Just as players might respond to the same cues differently when the team is ahead or behind, so, too, cellular responses to signals reflect the stage of development of the organism, the tissue and context in which the cell is found, what other proteins are already present in the cell, and so on. We can also invoke parallels to umpires, referees, and rulebooks, as many signaling networks include components that put careful checks on the system, with stoppage of play interrupting the flow now and then to ensure that things are done safely and the rules of the game are followed.

Having once imagined the situation this way, we next have to extend our thinking and consider that the cell is playing multiple, concurrent games all on the same field, with many signals received and transduced by a cell at the same time. To have several signal transduction pathways active at once is the cellular equivalent of hosting a softball game on the same field on which other players are playing field hockey or soccer. Keeping things in order takes coordination. Subsections of the field might be sectored off,

and what is happening in one game might be monitored by the coaching staff of another. Consistent with this idea, researchers have found that some proteins involved in transduction of specific signals are cordoned off into discrete subregions of the cell, and others serve as integration points for more than one signal.

Writing the Prequel to Embryonic Development

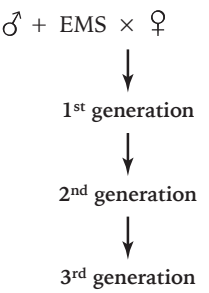
As mentioned, a goal of the Heidelberg screen was to "saturate"—to find all the genes for which a disrupting mutation can lead to the phenotype the researchers were looking for. One indicator that they had accomplished this goal was that they uncovered two or more mutant alleles for most of the genes identified in the screen via their mutant phenotypes (Nüsslein-Volhard and Wieschaus 1980). Nevertheless, there seemed to be a numbers problem. Despite the complexity that must be involved in building a properly organized larva, the total number of genes reported in 1980, "mutations at 15 loci" (fifteen genes), was small (St Johnston 2002; Nüsslein-Volhard and Wieschaus 1980).

Surely there were more genes involved.

But, very likely, a different strategy would be needed to find them.

By mass, the chromosomes make up a tiny portion of the egg. The rest of the egg comprises molecules provided by the mother that will support the early moments of fly life—fats, proteins, RNA molecules. The different strategy successfully undertaken by *Drosophila* researchers to find more genes was based on the underlying assumption that, whereas some of these molecules are just building blocks, some of the RNAs and proteins packed into an egg by the mother might serve as pre-loaded information molecules required to define the axes of the larval body. As these information molecules are provided by the mothers, not made de novo by the embryo, it follows that for this endowment to be complete, the female that made the egg must be wildtype for the genes that encode these gene products. If one of these genes is mutant in the moms, then the resulting embryos will lack that normally maternally provided RNA or protein during their early development, and cannot progress normally, even if their own copies of the gene are normal.

Various lines of evidence had suggested already that such "maternal-effect" factors would be present. To test this assumption and identify the relevant genes, T. Schüpbach and E. Wieschaus performed "maternal-



The maternal-effect screen strategy. This strategy extends by one generation the original Heidelberg screen strategy. Homozygous second-generation females are collected and crossed to wildtype males. The larval cuticles of the third generation are then examined for defects in organization.

effect screens," in which they started by making homozygous mutant fly moms, then looked for defects in the larval cuticle patterns of their offspring (Schüpbach and Wieschaus 1989; 1991). As predicted, using this approach, the researchers identified several additional genes required for making a properly organized larva, revealing additional components to define the dorsal-ventral axis. (Although not involved in establishment of the larval cuticle pattern, as shown by B. Wakimoto and colleagues, there are, in addition to many maternal-effect genes, a few "paternal-effect" genes, genes that must be wildtype in fly dads in order to kickstart very early events, such as fertilization of the egg by the sperm [Fitch et al. 1998].)

With another approach, researchers extended this reasoning but added an additional assumption: that for some genes required as maternal factors, disruption of the gene would cause lethality, such that a female homozygous for a mutation in the genes would never survive, and thus could never be tested in a standard maternal-effect screen. To get around this problem, N. Perrimon and colleagues developed methods for making the germline cells (the presumptive eggs) mutant, while keeping the rest of the fly wildtype. The trick combined making "mosaic clones" (Chapter 4) in the female germline with what is known as the "dominant female sterile" approach, which renders it impossible for a female to make eggs other than the homozygous mutant eggs the researchers want to examine (Perrimon, Engstrom, and Mahowald 1984; 1989; Perrimon et al. 1996). Together, the results of these two types of maternal-effect screens helped fill out the list of genes required to make a normal larva with a head at the head end, tail at the

back end, and with all the right segments in between. As was true for genes identified in the Heidelberg screen, when these genes were identified at the DNA sequence level, striking similarities to human proteins were again recognized.

Caught and Carried

The Hh protein is released by one set of cells, moves some distance away, and is received by cells also some distance away, inducing change in these recipient cells. These properties of Hh are in common with the properties developmental biologists proposed would be true of a morphogen, including the ability to create a gradient of activity. The different levels of morphogen activity experienced by cells close to the source (with more Hh binding to more receptors, activating more intracellular transducers) leads to different outcomes as compared with cells farther from the source of the activity. But extracellular space is not the vast vacuum of outer space. How does Hh travel? Does it diffuse freely? Or is its movement restricted, controlled?

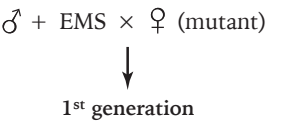
To set up a gradient, Hh and other signaling ligands are not allowed to diffuse freely, happening upon receptors by chance. Morphogen gradient-forming ligands like Hh travel from their source in a more controlled fashion, helped along like a crowd-surfer, or shuttled like a celebrity into a vehicle that will take her where she needs to go. One of the genes identified in maternal-effect screens sheds light on what happens to ligands and receptors at the surface of cells. The gene, identified by N. Perrimon and colleagues, was named *tout velu,* French for "all hair," as the cuticles of larvae that result when the female germline is mutant for *tout velu* look similar to the *hh* mutant phenotype, with the cuticle largely made up of hairy denticle belts (Bellaiche, The, and Perrimon 1998). When they identified the sequence of the gene, Perrimon and colleagues realized that *tout velu* encodes a member of the "proteoglycan" family of proteins. These proteins could be described as decorated like a fancy cake with frosting, with branchlike extensions made of linked sugar molecules extending from the protein core. A few years before *tout velu* was identified in *Drosophila,* disruption of a related human protein, EXT1, was implicated in a disorder associated with over-proliferation and the development of bone-related cancers called hereditary multiple exostosis (Hecht et al. 1995). The fly data helped to build new bridges, with an intellectual connection made between EXT1 and Hh signaling.

This finding was built upon a "paradigm shift" (Turnbull 1999) that began with a 1991 report based on research in cultured mammalian cells suggesting that heparan sulfate proteoglycans interact with the FGF signaling ligand (Yayon et al. 1991). Implication of proteoglycans in signaling in mammalian cells and *Drosophila* revealed that these sugar-decorated proteins, and likewise the proteins required to make the sugar chains and attach them, are more than just parts of the structural undergrowth of the cell surface. Through this "wealth of new and exciting information about proteoglycan function . . . revealed through the application of genetic approaches" (Turnbull 1999), proteoglycans came to be recognized as important components of conserved signaling systems. Indeed, though the precise mechanisms differ, these sugar-decorated proteins have in common an influence on the shape of the gradients of responses generated by various signaling ligands and their receptors (Yan and Lin 2009).

When What Goes Right Goes Wrong

As the molecular identities of components of the Hh pathway and other signal transduction pathways were uncovered, researchers began to recognize the striking conservation of signal transduction components in other organisms, including not just the presence of similar genes in these organisms but also a commonality in the way the signaling networks are wired up, with one protein acting on another in a defined order preserved among species. Related to this, molecular identification of the genes revealed that many of them have orthologs in the human genome, and that some of these human genes had been implicated in cancer or other proliferative disorders. Various human orthologs of signal transduction pathway proteins uncovered in the fly and other model systems were noted as highly active or expressed in high levels in some cancers, or as altered in sequence in patients with inherited diseases associated with high rates of cancer or other disorders associated with an excess of cell proliferation.

A convergence of understanding was emerging—signaling genes important for normal development were related to genes whose disruption caused cancer, and vice versa. In 1991, M. Simon, G. M. Rubin, and colleagues used a mutation in a signaling pathway in a "dominant modifier" screen to find "enhancer" or "suppressor" mutations, which make the mutant phenotype associated with disruption of a gene worse or better. Among other genes, the group identified a fly ortholog of the human cancer-associated *RAS* gene (Simon et al. 1991). Other genes newly identified in the 1990s as



A dominant modifier screen strategy. Mutagenized males are crossed to females carrying a dominant mutation. The first-generation progeny are examined for flies in which the dominant mutant phenotype is made better or worse in the presence of an additional mutation.

relevant to signal transduction in *Drosophila,* including *Son of sevenless* (*Sos*) and *corkscrew* (Pierre, Bats, and Coumoul 2011; Perkins et al. 1996; Perkins, Larsen, and Perrimon 1992), were also found to have human orthologs with cancer-related roles. Understanding this connection led to additional discoveries. Researchers began to use human cancer genes as a starting-point to find more fly genes relevant to signaling and development. Various groups demonstrated in the 1980s and 1990s, for example, that there are orthologs present in the *Drosophila* genome for already well-known human cancer genes, including *RAF* (Kolch et al. 2002; Mark et al. 1987), *SRC* (Simon, Kornberg, and Bishop 1983; Hoffman-Falk et al. 1983), and *ABL* (Hoffman-Falk et al. 1983).

Signaling Proteins as Cancer Drug Targets

Perhaps most important, with a new understanding of what underlies cancer at the cellular level—with disruption of communication as a key aspect of the disease—as well as new knowledge regarding what specific proteins are involved, we gain new ideas regarding the subcategorization of cancers into types and the development of treatments. An understanding of the functions of the proteins in a pathway is essential to identification of appropriate "drug targets" for which we can seek out drugs that inactivate function; using a drug to inactivate a protein that functions as a positive regulator of a given signaling pathway—passing the signal along—will have a very different outcome from inactivating a protein that acts as a negative regulator. The *smo* gene was so-named because the cuticles of mutant larvae lacked ventral structures—a phenotype that is opposite the hairy *hh* mutant effect, and reflects the opposite role of *smo* versus *hh* in Hh pathway signaling. It stands to reason, then, that treatment with drugs that inactivate Hh-related proteins will have different effects compared with drugs that inactivate the human SMO protein. As it turns out, tar-

geting SMO has proved to be an effective strategy for treatment of some cancers.

Several inhibitors of SMO have been approved for use in humans as chemotherapeutics for specific subtypes of cancer (Takebe et al. 2015). The first of these to be approved by the U.S. Food and Drug Administration (FDA), approved in January 2012, was vismodegib (Nix, Burdine, and Walker 2014), which has been described as displaying "remarkable effectiveness" in the treatment of basal cell carcinoma (Atwood, Whitson, and Oro 2014). Another SMO inhibitor, known as glasdegib or PF-04449913, has been explored in clinical trials as a possible treatment for some types of cancers and other disorders of "myeloid" or bone marrow cells (Martinelli et al. 2015). Interestingly, some SMO inhibitors originated from studies of the natural products of plants, as is the case for cyclopamine (Hovhannisyan, Matz, and Gebhardt 2009). Cyclopamine was first identified via developmental defects noted in offspring of animals pastured in fields in which the plant *Veratrum californicum* was growing. As the name of the compound implies, among the most dramatic of these defects was the observation of cyclopia among lambs born from sheep that had eaten the plant. This developmental disruption helped researchers deduce that a compound produced by the plant was exerting an effect on Hh pathway signaling, and eventually led to explorations of the plant compound and related compounds as cancer therapeutics (Hovhannisyan, Matz, and Gebhardt 2009). For different cancers, different approaches will be appropriate; additional therapeutic strategies being explored include targeting the Hh ligands, as in the case of robotnikinin (Stanton et al. 2009), and targeting GLI proteins, downstream targets of Hh signal transduction related to the *Drosophila* Ci protein (Lauth et al. 2007).

Information learned about other signaling pathways and their study in the context of fly development similarly aids in the identification of new drug targets and treatments for cancers associated with disruption of those pathways. Identification in *Drosophila* of methotrexate as an inhibitor of the JAK/STAT signaling pathway opened a door to the potential use of this relatively inexpensive drug in treatment of myelofibrosis, a cancer of the blood and bone marrow (Thomas et al. 2015). *Drosophila* has also been used in studies aimed at identifying new treatments for a type of thyroid cancer that is associated with mutations in the human signaling receptor RET (Das and Cagan 2010; Vidal et al. 2005), as well as in studies related to a proliferative disorder known as tuberous sclerosis complex that is as-

sociated with disruption of components of another signaling pathway found in both flies and humans (Housden et al. 2015). Targeting of the Hippo signal transduction pathway provides an additional example, as discussed in Chapter 4.

From Linear Pathways to Complex Networks

The components of a signal transduction pathway, such as the Hh pathway, are often depicted in a linear fashion, with one protein acting on the next in a positive or negative way, and in isolation within the sketched outlines of a cell, as if that cell is exposed to only one signaling ligand. This model is useful for conveying the basic wiring of a network: diagramming what domino pushes over what next domino, and so on, in the transduction of a specific signal. It is also, however, a gross oversimplification of what is really going on in our cells. In response to a signal, or its stoppage, some components of signal transduction pathways move from one cell compartment to another, with such a translocation reflecting or causing a change in the activation status of the protein. Proteins also form and reform multiprotein complexes in response to signals, building machines that perform specific functions and then dissociate when signals teeter out or change. Some proteins modify one another in response to signals, as when proteins called kinases "phosphorylate" other proteins, a kind of corsage-pinning of a phosphate molecule onto one protein by another, conversely, when proteins called phosphatases "dephosphorylate" or remove a phosphate molecule. In many cases, signal transduction leads to changes in activity of transcription factors. However, there is not a simple relationship between transcription factors and the genes whose activities they regulate. A gene often has nearby landing pads for more than one type of transcription factor, and the number and type of transcription factors that bind can have different effects on transcription of the gene.

Our ability to grasp, visualize, and explore the complexity of signaling systems has benefited from fresh ideas brought in from outside the usual realm of biology, including the study of networks and social interactions (Barzel and Barabasi 2013). Many now recognize that the interactions among proteins in signal transduction pathways—which include interactions that convey, amplify, or dampen the initial message being sent—have similarities to social networks in which persons can be classified as friends, foes, and so on. Moreover, as our understanding of gene and pro-

tein networks grows, we are forced to seek new ways to visualize them. Some day we might be able to take a virtual walk through computer-generated renderings of signaling events within a cell, allowing us to explore how the whole interconnected system is disrupted when we take a component away or dial up activity of another, as can be true in disease.

The relative simplicity of the fly genome, and the deep foundation laid through studies in the late twentieth century, contribute to our ability to define and explore the rich and integrated signaling networks that are at play within cells. We catalog the on switches, off switches, and rheostats within a cell, the responses they elicit, and the after-effects that amplify or dampen signals already sent. Each new level of complexity we uncover, each new connection, leads to more questions, to a need for further cataloguing, additional experiments. Complexity is first pared down, then built back up again, with genetic, cellular, and biochemical data contributing to ever-improving computational and visual models of what is going on when cells signal to one another and respond. We learn what is in common and what differs at different stages of development or in different tissues and organs, and across the span of evolutionary time. The results of the Heidelberg screen, integrated with a large existing body of work and all that has followed, has helped us understand cell signaling and signal transduction well enough that we can intercept the signals and treat cancers. With new tools, new assays, and building on this solid foundation, we can carry progress farther forward.

CHAPTER FOUR

SIZE

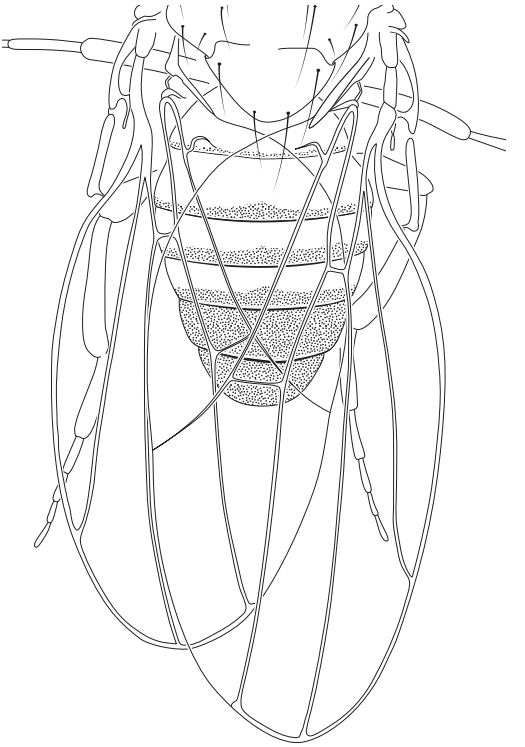
IF SMALL EQUALS CUTE, THEN SOME OF THE CUTEST *DROSOPHILA* ON the planet are the various strains of *Minute* mutant flies, the first of which were reported in the early 1920s (Bridges and Morgan 1923). In many of the 50 or so *Minute* mutant fly strains, there is one normal and one abnormal copy of a gene that encodes a component of the ribosome (Kongsuwan et al. 1985), a protein-making factory that is itself made up of a large number of gene-encoded RNAs and proteins. The ribosome reads the three-letter genetic code along the length of an RNA molecule and uses that information to build the corresponding protein from amino acids—the single units from which all proteins are made—by linking the amino acids together like cars on a train. When one normal copy of a ribosomal gene is missing, cells have less of that ribosomal gene product to incorporate into ribosomes, and thus end up with fewer protein-making factories. As a result, the overall rate of protein production slows, leading to the small size of the *Minute* mutant flies.

Impairing ribosome function is not the only way to make a small fly. Males are naturally smaller than females, for example, and environmental inputs come into play (Mirth and Shingleton 2012). Researchers recognized as early as the 1920s that flies raised at high temperatures tend to be

smaller (Alpatov and Pearl 1929), and limiting access of a fly larva to food will result in a small adult. Evolution also selects for differences in size over time. The largest *D. melanogaster* flies are those native to high-altitude Ethiopia; lowland-dwelling flies in nearby Zambia are considerably smaller (Lack, Yassin, et al. 2016). Regardless of the origin of their differences in size—genetic mutation, difference in temperature, natural selection of a local population—something these unusually small or large *Drosophila* have in common is that with few exceptions, all the structures normally present in or on a fly (wings, eyes, legs, and so on) are not only present, but also share the same relative proportions as in normal flies. Each tissue, organ, and appendage is scaled down or up in size to the same degree. The flies are not narrow-winged or big-headed, not squat or leggy. They are simply smaller or larger versions of the norm.

In 1915, T. H. Morgan reported the observation of a fly that breaks this rule of regular proportions (Morgan 1915). One half of the fly has the attributes of a male, such as presence of a "sex comb" on its forelegs (Chapter 6). Much of the other half, by contrast, has the attributes of a female, including a noticeably larger wing. The fly is a "gynandromorph"; it is made up of a mixture of cells, some with male identity and others with female identity, a condition that can result from an error in chromosome segregation during development. The existence of such a fly teaches an important lesson. We can learn from this fly that the sizes of the wings are controlled in a manner that has at least some degree of independence from what is going on in the whole: cells in each wing make different choices regarding when to stop growing, leading to different outcomes—two different sized wings—despite the fact that all the cells in this one fly experience the same environmental conditions and have equal access to nutrients.

Understanding how the final size of a complex multicellular animal is controlled, with all its component parts present in the right proportions, turned out to be an enduring problem, with major questions still unanswered at the beginning of the twenty-first century. By the 2000s, we knew that, given the right signals, cells can grow larger and proliferate (divide), or, conversely, stop growing and, in some cases, die. Moreover, we knew that in flies, growth stops when hormones trigger maturation, as a larva transitions to a pupa. What was largely missing, however, was a sense of how growth, an activity, is regulated to control size, the outcome, including



Part-female, part-male "gynandromorphy" fly observed in 1915. The left side exhibits male characteristics, including smaller wings as compared with the right side, which exhibits female characteristics. Redrawn from T. H. Morgan (1915), "The infertility of rudimentary winged females of *Drosophila ampelophila*," *American Naturalist* 49 (580): 240–250, Figure 2.

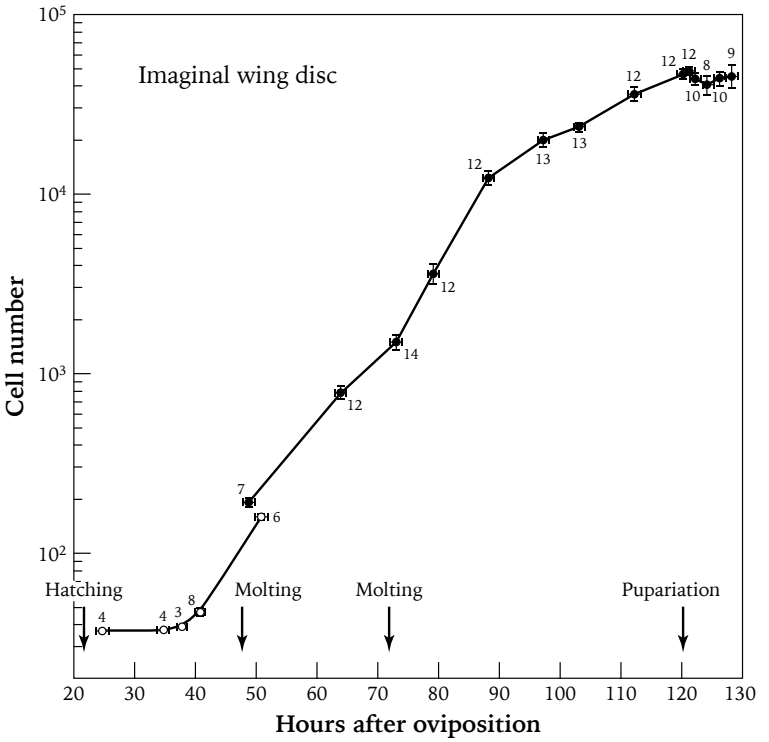
how the size of individual appendages and organs are kept in proportion. Results uncovered first in *Drosophila* provided an answer, revealing a new signal transduction pathway that acts in size control—a new set of players on the field, playing a new game, one not identified in previous studies. As a result of this new information, researchers were able by 2012 to say that "the field of size control finds itself in the fortunate position of finally being able to tackle century old questions of how organisms achieve final adult size and proportions" (Mirth and Shingleton 2012).

What was so special about *Drosophila* that it was in this species that experimental work provided an answer to a long-held biological question?

Growth of a Fly Larva

An adult fly is corseted by its exoskeleton, which leaves little or no room for growth. Thus the dramatic increase in mass associated with the transition of a tiny fly embryo into a full-sized adult must take place during the larval stage. Larval growth is so dramatic that more than once the fly larva "molts" or sheds its cuticle, each time loosening the bounds of a skin that has grown too tight. After the third and final larval molts, hormones trigger a different reaction, inducing the larva to crawl out of the mush of its food source and seek higher ground, during what is known as the "wandering" larval stage, and then form a pupa. Cells within a larva can be divided into two types. Most larval cells will be "histolyzed" or degraded in the pupa, reduced to a nutrient soup that feeds formation of the adult (the "imago"). But specialized cells set aside in the embryo, the "imaginal" cells, which are organized in larvae into pairs of wineskin-shaped "imaginal discs" or more loosely formed imaginal clusters, grow during larval stages, stay intact in the pupa, and, as metamorphosis occurs within the pupa, form the adult structures (Beira and Paro 2016). There are nine pairs of discs in the larva, which will give rise to the pairs of eyes, legs, wings, and so on, as well as a single disc that will form the genitalia.

From decades of research, we have a good sense of where hormones are produced in insects (Appendix B), what hormones are made, how they act within cells, and when. This work included testing the effects of various fine-scale surgical interventions. Insect physiologists tied off subregions of larvae in an effort to locate sources of hormones; removed hormone-producing glands or the brain from insects of various stages, then assessed the subsequent impact on growth; and implanted tissue from an insect at one stage of growth into the body cavity of an older or younger host to test the capacity of host hormones to affect the implanted tissue. V. B. Wigglesworth even grafted two larval or adult assassin bugs (*Rhodnius*) together at their necks, then observed the effects on the conjoined insects, again to learn about hormonal control (Wigglesworth 1965). Researchers also measured growth. In the *Drosophila* field specifically, painstaking work was done to quantify the proliferation of imaginal cells, so as to dissect the wing imaginal discs from embryos and larvae of different stages and count—by hand, aided by a microscope—the number of wing imaginal disc cells present at different stages of development, a number that exceeds 10,000 cells in larvae (Bryant and Simpson 1984). This rapid growth ceases with



Growth curve of the *Drosophila* wing disc. Growth is expressed as the mean number of cells per wing imaginal disc and shown on a logarithmic scale. Reprinted from P. J. Bryant and P. Simpson (1984), "Intrinsic and extrinsic control of growth in developing organs," *Quarterly Review of Biology* 59 (4): 387–415, Figure 3. © 1984 by the Stony Brook Foundation, Inc., with permission from the University of Chicago Press and Pat Simpson.

the maturation associated with reaching the pupal stage—an effect opposite of that observed for us humans, in which maturation into an adult is associated with a growth spurt (Boulan, Milan, and Leopold 2015). Through microsurgical and transplantation studies, *Drosophila* researchers also learned that imaginal discs have the capacity to regenerate, with cells proliferating to restore normal size following partial removal of disc tissue (Bryant 1971; Schubiger 1971).

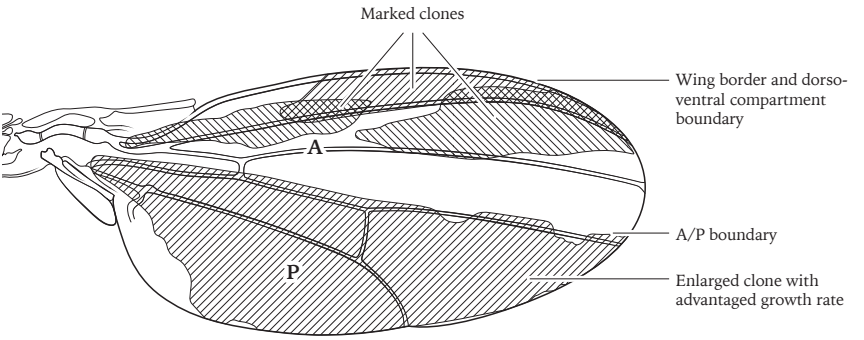
But, as discussed, growth is one thing, and size is another. Even steroid hormones, which circulate throughout the developing insect, do not help us understand the different-sized wings of the gynandromorph. The two wings of the fly were exposed to the same hormones, at the same levels,

and at the same times, yet they still grew to different final sizes. Something intrinsic—acting within the cells—must also be at play, controlling final size. There were hints in the twentieth century that genetics could help reveal aspects of growth and size, as in isolation of the various *Minute* mutant fly strains or of the mutant fly strain known as *lethal (2) giant larva,* which was reported in 1937 (Hadorn 1937). Many other growth-relevant genes were isolated through identification of mutant strains that are smaller or larger in size, as in the case of the *chico* mutant flies, which disrupt insulin signaling (discussed below). Tackling the problem of proportional size, however, presents a special case and would require additional genetic tools.

Islands of Difference

The gynandromorph tells us something about the genetic control of size. But it also tells us something else. It exemplifies the fact that we (or in this case, a natural accident) can create a "genetic mosaic," an animal made up of cells of more than one genotype. Researchers had long recognized that mixing different genotypes of cells can be exploited as a tool in experimentation. One way to do this is to transplant tissues from one individual to another. Classical embryology studies include production of two-headed frogs via transplantation of tissue from a donor embryo into a recipient, as well as mixing cells from two different species of bird, which allowed researchers to track the contributions of donor cells to various final structures. A problem with these approaches, however, is that they involve microsurgery—careful dissection of subregions of embryos that are already quite small, followed by their transplantation into a host. These studies revealed much, but they cost a lot in time and resources, and were not practical to use in larger-scale studies, such as in a genetic screen.

The identification of gynandromorphs and other naturally occurring genetic mosaic animals shows us that a mixture of genotypes can be achieved not only through physical transfer of cells from donor to host, but also through genetic manipulation within a single organism. The ideal genetic system for making mosaic animals would allow for careful control, such as the ability to make a cluster of mutant cells within a field of otherwise normal cells, or to alter all cells of a given tissue or organ, again within the context of an otherwise normal fly. Through the study of atypical chromosome events, researchers recognized that somatic recombination, which is



Mosaic clones that arose from X-ray induced recombination. Drawings of clones that arose from X-ray–induced mitotic recombination in wing discs of two-day-old *Drosophila* larvae. A, anterior region of the wing. P, posterior region. Outlines of clones from different individuals are superimposed. The large clone is composed of cells with a higher growth rate than in the rest of the disc. Redrawn from P. J. Bryant and P. Simpson (1984), "Intrinsic and extrinsic control of growth in developing organs," *Quarterly Review of Biology* 59 (4): 387–415, Figure 4. © 1984 by the Stony Brook Foundation, Inc., with permission from the University of Chicago Press and Pat Simpson.

similar to crossing over as described in Chapter 2, can create a genetic mosaic in which a group of cells are homozygous for a mutation in an otherwise heterozygous animal. Such an island of mutant cells is commonly referred to as a mosaic "clone" of mutant cells, and a study exploiting the approach is called a "mosaic analysis" or "clonal analysis."

In the earlier years of *Drosophila* research, X-rays were used to make mosaic clones, as the double-strand DNA breaks induced by X-rays can lead to somatic recombination. This approach was effective but not efficient; even following treatment with X-rays, mosaic clones were rare. In 1989, K. Golic and S. Lindquist reported an easier, more controllable, and more efficient approach based on a system from yeast in which a protein (FLP) efficiently induces somatic recombination at a specific DNA sequence (FRT), which can be inserted into flies using transposons (Golic and Lindquist 1989). The Gal4-UAS system (Chapter 2; Brand and Perrimon 1993) gave researchers additional control. This system facilitated induction of FLP in specific tissues, allowing researchers to control in what cells mosaic clones are produced, and, when combined with RNAi or CRISPR technologies, made it possible to alter gene function in Gal4-expressing

tissues, an effect similar to making a mosaic clone. With the toolkit expanded, new types of experimentation followed.

In 1995, two groups showed that production of clones of cells in the fly eye in which the gene *warts* has been mutated results in overgrowth (Justice et al. 1995; Xu et al. 1995), and in 2002, the results of two studies pointed to a similar outcome for mutations in the gene *salvador* (Kango-Singh et al. 2002; Tapon et al. 2002). The next year, the I. K. Hariharan and D. Pan labs reported a third gene, *hippo,* that results in a similar mutant phenotype (Harvey, Pfleger, and Hariharan 2003; Wu et al. 2003). Based on the mutant phenotypes resulting from *warts, salvador,* and *hippo* mutant clones, as well as characterization of cell division and cell death in these clones, researchers deduced that the normal function of these genes is to restrict size. Indeed, these reports led to the identification of a new signal transduction pathway, the Hippo pathway, that requires *warts, salvador, hippo,* and other genes, and has the ability to coordinate cell growth, proliferation, and death (Pfleger 2017). When the *hippo* gene function is depleted in a tissue, such as in the eye, that tissue becomes overgrown: the checks on growth and compensatory cell death that would normally balance size are absent. At a molecular level, Hippo protein, which is activated or inactivated in response to changes in cell density, acts through additional proteins to affect the activity of a transcription factor, Yorkie, which controls the on-off status of relevant genes. Crosstalk of the Hippo pathway with other pathways further influences size-related outcomes. As expected for a system controlling size, control by Hippo differs from control by growth hormone signaling; rather than responding to a systemic factor, Hippo signaling depends on local cell contacts, such that the Hippo pathway can influence the size of an organ or tissue independent of what is going on elsewhere in the organism.

Because the availability of genome sequence made finding similar genes in different organisms much faster and easier in the early 2000s than it had been when genes like *hh* were first identified, as soon as the *warts, salvador,* and *hippo* genes were sequenced, researchers could do a quick computer check and see that orthologs are present in the human genome. As has become less surprising now than in the past, researchers quickly found, too, that these similar proteins encoded by mammalian genomes are wired up with one another in similar ways, and that in other organisms, as in flies, the Hippo pathway similarly controls the balance between cell proliferation and

cell death, and hence size. Exemplifying the high degree of similarity between Hippo proteins in the fly and in mammals like ourselves, a study published in 2003 showed that the human *STK3* gene (also known as *MST2*) can stand in for *hippo*, restoring the normal cell balance when introduced into a *hippo* mutant fly (Wu et al. 2003). Other types of studies in the fly, such as using known Hippo pathway proteins as bait to fish for additional components in the pathway (Kwon et al. 2013), are further expanding our knowledge of Hippo signaling, including its interactions with other pathways and cellular functions.

Comparing fly genes in the Hippo pathway with human genes also revealed connections to disease. Consistent with a role for the pathway in keeping size in check, mutations in several human orthologs of fly Hippo pathway components are associated with cancer. Increased copy number or activity of the human gene *YAP1*, an ortholog of *yorkie,* is associated with a variety of cancer types (Moroishi, Hansen, and Guan 2015). Human orthologs of *hippo* and *crumbs* are "tumor suppressor genes"—when the genes are functional, things are fine, but when their function is disrupted, such as through mutation or deletion, the mutant cells proliferate. Mutations in human orthologs of *fat* are associated with cholangiocarcinoma and a specific type of leukemia; mutations in the human ortholog of *four jointed, FJX1*, are associated with colorectal carcinoma; and mutations in other conserved Hippo pathway genes have been found in specific types of renal, prostate, breast, ovarian, and cervical cancers (Ye and Eisinger-Mathason 2016). For some of these genes, biomedical data had already suggested that the genes were relevant to cancer. By placing them in the Hippo pathway, we gained a sense of how they are relevant and how we might approach therapeutic treatment (Johnson and Halder 2014).

Winner Takes All

Mosaic clones have taught us about size in other ways as well. In the experimental generation of a mosaic clone, researchers use genetic tricks to create two cell populations, one in which the mutation is present, and a second, "twin" population that is wildtype. If the mutation has no effect on cell growth, then cells in the mutant clone and its twin will divide and grow equally, such that the relative sizes of the two cell populations will be about the same. Researchers have long observed, however, that not all mutant clones reach the same size as their twins or compete equally with sur-

rounding cells. Clones of *Minute* cells, for example, do not populate the same final space as their twins. This is not too surprising. *Minute* mutant cell clones affect protein synthesis, such that cells in a *Minute* clone are slower-growing. More surprising, however, is the observation that *Minute* clones sometimes disappear altogether, or grow to a size that is smaller than what would be predicted based on the relative rates of division of *Minute* and normal cells (Morata and Ripoll 1975). Research in *Drosophila* investigating this and related phenomena led to the first evidence of "cell competition," in which cells with a fitness advantage relative to neighboring cells survive, whereas their less fit neighbors undergo cell death (Morata and Ripoll 1975; Moreno, Basler, and Morata 2002; Simpson 1979), as observed not only for *Minute* mutant clones but also for clones of cells mutant for a gene called *Myc,* also known as *diminutive* (Johnston et al. 1999).

In 2012, S. de Beco, M. Ziosi, and L. A. Johnston noted that "field of cell competition has seen a huge explosion in its study in the last several years" (de Beco, Ziosi, and Johnston 2012). Most of the foundational studies in this area were performed using fly wing imaginal discs and mosaic techniques such as the FRT-FLP system. We now know that cell competition is context-dependent, kicking in only when neighboring cells exhibit differences in fitness, not when all cells are affected. The process also requires direct contact between would-be "winner" cells and "losers" (Simpson and Morata 1981). In at least some cell types or contexts, cell competition in *Drosophila* depends on a gene called *flower* (Casas-Tinto, Torres, and Moreno 2011). Most cells express one isoform (version) of *flower,* but weaker cells—those fated to die—express different isoforms, known as loser isoforms, that activate different sets of downstream signals within the cell (Casas-Tinto, Torres, and Moreno 2011). We also know that cell competition is required for normal development, as mutations in the gene *azot,* which is required for elimination of loser cells, results in flies with morphological defects such as blisters and nicks in the wings (Merino et al. 2015).

Since its initial discovery in flies, researchers found evidence of cell competition among mammalian cells as well (Penzo-Méndez and Stanger 2014). As in flies, in mammalian cells, this activity requires cell-to-cell contact and induces apoptotic cell death in losers (Penzo-Méndez et al. 2015; Penzo-Méndez and Stanger 2014). Why would cells have evolved such a mechanism? The rationale behind the "social cellular phenomenon" of competition (de Beco, Ziosi, and Johnston 2012) might be likened to the

idea that weaker players on a team are cut or convinced to step aside, acknowledging that the team's chances would be better if resources are focused on more talented players. In a normal tissue, this would help the organism reach a healthy size and survive, such that cell competition could be characterized as a system of organized self-sacrifice. M. Amoyel and E. Bach have also speculated that cell competition might be a remnant of adaptation of unicellular organisms to multicellularity (Amoyel and Bach 2014). Moreover, in both flies and mammals, genes with roles in cell competition help regulate growth and regeneration (Gogna, Shee, and Moreno 2015; Penzo-Méndez and Stanger 2014).

As for other activities associated with development, growth, and size, misregulation of cell competition has been linked with cancer. Indeed, the cellular mechanisms that control cell competition are exploited by cancers in a way that favors the more aggressive (and thus more dangerous) cancer cells, at the expense of normal cell survival. Inactivation of normally growth-restricting Hippo signaling pathway components, such as mutation of the *hippo* gene, appears to promote cell competition, such that *hippo* mutant cells are able not only to outpace their neighbors with regard to growth rate but to eliminate them altogether (Saucedo and Edgar 2007). A similar effect is observed in mosaic clones of fly or mammalian cells expressing high levels of MYC (de la Cova et al. 2004; Claveria et al. 2013). Another conserved gene, *SPARC*, also has demonstrated roles in cell competition, and mutations in the gene are associated with several cancer types (Gogna, Shee, and Moreno 2015). L. Baillon and K. Basler stated in 2014 that "developmental programs constitute the toolbox from which cancer cells help themselves to promote and sustain their malignancy." They go on to suggest that "misregulation of cell competition represents a tool of choice in this process" and that further elucidation of cell competition will be "best achieved" in *Drosophila* (Baillon and Basler 2014). Underscoring the importance of the work, cell competition has been implicated not just in cancer but also in immune system, heart, and neurological diseases (Gogna, Shee, and Moreno 2015).

Insulin, Diabetes, and Size

In addition to mutation of *Minute* genes, there are other ways to make a small fly through genetic manipulation. One of these is to disrupt genes involved in hormone signaling by insulin, as in *chico* mutant flies, which are

less than half the size of their wildtype siblings (Bohni et al. 1999). Insulin is a peptide—a short protein, encoded by a gene—that in humans is secreted by specialized cells in the pancreas called islet cells. A basic function of the insulin hormone is to control absorption of sugar by cells. When insulin levels rise in a healthy person, cells are stimulated to take up sugar, and thus the concentration of sugar in the blood goes down. Particularly given the increase in diagnoses of type II diabetes in the United States and other countries, *Drosophila* researchers have placed new emphasis on the study of insulin and its effects at the whole-organism, tissue, and cellular levels. Though much about insulin signaling is already known through mammalian studies, the entry of *Drosophila* research into the field has provided some fresh perspective. Some of these studies focus on insulin signaling itself; others relate to the study of obesity, which increases the risk of type II diabetes.

Drosophila insulin-like peptides (Dilps) are made by a specialized set of cells called insulin producing cells (IPCs) located in the fly brain. Like human insulin, Dilps are gene-encoded peptides that regulate the fly's growth and energy balance. When stained with a fluorescent marker, the Dilp-producing IPCs light up, appearing against the dark field of the micrograph like clusters of balloons, with string-like extensions dangling down from their round cell bodies, though the "strings" are less like passive objects and more like tentacles, creeping down from the cell bodies and making purposeful connections along the extents of their paths. Putting a fly on a high-sugar diet leads to changes in the expression and accumulation of Dilps in the IPCs and, eventually, to phenotypes with marked similarities to diabetes. Through extensive work, including the experimental definition of an extended *Drosophila* insulin-signaling network of more than 500 proteins (Vinayagam et al. 2016), much is now known about insulin signaling and nutritional responses in the fly, laying an important foundation for further study and facilitating comparison with the more complex human system of insulin regulation and nutrient responses. Studies have also suggested that differences in insulin signaling contribute to the difference in body size between male and female flies (Rideout, Narsaiya, and Grewal 2015), with genes on the X chromosome also exerting control (Mathews, Cavegn, and Zwicky 2017).

One of the areas in which insulin-related studies in *Drosophila* can give a boost to biomedical efforts is in the prioritization of genes relevant to diabetes or obesity. Whereas many genetic diseases can be traced back to

disruption of one or a few genes, researchers estimate that differences in any of some 250–350 human genes—1 percent of all human genes—might contribute to a person's propensity to become obese (Yoneyama et al. 2014; Speliotes et al. 2010). Using various approaches, experts in human genetics have identified dozens of candidate obesity- and diabetes-related genes. But finding a candidate and knowing for sure that a gene is definitely involved are two different things. Moreover, even after a gene is causally linked, exactly what the connection is might not be apparent. The products of some obesity-related genes, for example, might affect our cellular metabolism, causing all the cells of the body, or subsets, to burn more or less energy; others might affect our brains, triggering hunger or satiety.

Sorting out which genes affect which processes, and how, is important to further progress. In this area, as others, we can leverage *Drosophila* as a platform for rapid genetic analysis. An example is a study reported by R. L. Cagan, F. S. Collins, T. J. Baranski, and others in which mutation of the fly ortholog of a human gene, *HHEX*, which was identified in a genome-wide association study focused on type II diabetes, was found to result in changes to sugar levels and insulin signaling in the mutant flies, providing strong evidence that this candidate is involved in similar processes in humans (Pendse et al. 2013). Studies in flies have also helped identify the relationship between obesity and gene function for the human gene *NUDT3*, which appears to be involved in regulation of insulin signaling (Williams et al. 2015). In summarizing their own work, J. Pendse and colleagues state that "Candidate genes identified in human genetic studies of metabolic traits can be prioritized and functionally characterized using a simple *Drosophila* approach" and that the "most important advantage" of using *Drosophila* in this type of study "is the ability to assess all candidates in an unbiased manner, identifying surprising hits and untangling complex regions" (Pendse et al. 2013). Whether motivated by human genetic studies, as in these cases, or initiated in the fly, as for *hippo,* the small size of the fly belies its potential to have a large impact on our understanding of fundamental biological concepts.

CHAPTER FIVE

DIRECTION

THE PARADISE ROCK CLUB, A MUSIC VENUE IN BOSTON, MASSACHUSETTS, has a simple layout. There is a bar tucked off to one side, a platform stage, and an otherwise open floor, surrounded on three sides by a balcony. Every show at the Paradise is general admission; there cannot be assigned seating, as there are no chairs. Before a band takes the stage, someone watching from the balcony could discern little if any sense of order. Fans stand around, distributing themselves in small groups, perhaps walking over to the bar or to a table, usually set up opposite the bar, where roadies sell screen-printed t-shirts and vinyl records. When the headlining band takes the stage, however, everything changes. Fans turn to face the stage and squeeze in close to one another, eventually forming a single pulsing mass, each person a part of a coordinated whole. By the time the band launches into a crowd favorite or extended solo, each person, each fan's face, is focused intently; each body leans in, taking in the sound. Some in the crowd might extend a single arm toward the stage, smartphone in hand, to take a picture or video, their feet still anchored to the floor, bodies moving to the beat. At the end of the evening, when the band has finished the final song of its final encore and the scene is suddenly bathed in bright overhead light, fans reorient, turning from the stage to the exit. They file out, acting one last time as a coordinated collective before they reach the exit to the

wide Commonwealth Avenue sidewalk. There, various small groups and individuals split off and scatter. Some cross the street to the Green Line trolleys. Others stand around waiting for rides or walk to their cars. No trace remains of the ordered orientations that were visible just minutes before.

There is a beautiful parallel between what happens when a band takes the stage at a small-venue concert hall, each eager fan facing the musicians, and the organization of the fine sensory hairs within our ears that detect sound, transducing the mechanical force of a soundwave into something we perceive as music, speech, birdsong, tires on wet pavement, the roaring sea. The sound-sensing region of the inner ear is comprised of an "epithelium," a sheet of cells. Within the epithelium, "support cells" surround the specialized "sensory cells" that do the business of detecting sound. Like so many fans pointing smartphones at the band to take photos, the sensory cells extend microscale radio towers called "kinocilia" and "stereocilia" that are stimulated by sound and transduce this auditory information via neurons to the brain.

This organization of a sheet of cells, with some cells singled out as special and with specific structures oriented relative to some axis, is by no means exclusive to the vertebrate inner ear. The phenomenon is reflected, also, in the ordered orientations of the hairs on our forearms, and in the positioning of hairlike cilia on the epithelia of the lung, the spinal cord, the heart, and the kidney, where the pulsing movement of these cilia—all oriented in the same direction—brings about the directed flow of mucus (in the case of the lung), cerebral spinal fluid (in the case of the spinal cord), and so on.

We can ask, what if anything does the organization of sensory cells in our inner ears and the directed positions of kinocilia within them have to do with a fruit fly? The answer lies in the mechanisms by which cells in an epithelium take on this directed organization. Biologists call this phenomenon—the cellular equivalent of fans orienting to the stage—"planar cell polarity," or PCP. We know a lot about how PCP works at the level of cells and molecules. We would probably still be struggling to understand even the basic principles of this phenomenon, however, if not for the driving curiosity of researchers interested in a similar phenomenon in the fly: the planar pattern of hairlike sensory bristles on the fly's wing. Through work done in the 1990s, the molecular mechanisms of PCP were uncovered. It was work "largely ignored" at the time by others (Carroll and Yu 2012).

But these researchers eventually succeeded in revealing a "clear, detailed, and extensive" set of mechanisms (Eddison, Le Roux, and Lewis 2000) that were later demonstrated to underlie how PCP is established not just in the fly but in our cells as well, including in the inner ear.

Learning about PCP

Interest in PCP in *Drosophila* arose at least in part from studies of this phenomenon in the milkweed bug (Lawrence 1966) and other insects in the 1950s and 1960s (Wehrli and Tomlinson 1995). In insects, PCP is readily evident in the curve of the bristles on the outer cuticle, always leaning toward the posterior end, and in the eye, wherein an asymmetry within the component units of the eye, the ommatidia, takes on a regular orientation along the anterior-posterior and dorsal-ventral axes. As mentioned, PCP is also readily evident in the insect wing. The fine wing hairs, which extend like tiny tent-poles under the covering sheet of the wing's thin transparent cuticle, always bend toward the distal tip. To understand the phenomenon, we can walk backward in developmental time. Early in the formation of a wing, cells peppered at regular intervals within the epithelium are tapped to become the wing hairs (discussed later in the chapter). After these cells take on a wing hair-cell fate, there remains a need for the cells to learn where they are along the plane of the epithelium. Telling up from down is easy. The "basal" or bottom surface is defined by the anchoring of the cells in the sheet to some surface, typically an "extracellular matrix," secreted by cells underneath the epithelium. By contrast, compass points across the sheet—in the planar axis—are not self-evident. How cells know where they are along the proximal-to-distal axis of the wing was an intriguing mystery that sparked the interest of fly biologists, who believed they could apply a genetic approach to understanding the phenomenon.

One reason *Drosophila* has been particularly valuable in PCP research is that it is relatively easy to generate mosaic clones of mutant cells in an otherwise wildtype fly (Chapter 4). Important to research into PCP, mosaic analysis also allows researchers to distinguish between two types of gene activity. Cell-autonomous activity can be defined as a requirement for gene activity only in the same cells in which the gene is turned on, as would be typical for, say, genes that encode enzymes that act within the cell's cytoplasm or signal transducers that act inside the cell in which they are made. Non-cell autonomous activity, by contrast, can be defined

as a requirement for a gene product in cells other than the cells in which the gene is expressed. This typically reflects secretion of a protein by the cells that produce it and a subsequent induction of some new activity in neighboring cells exposed to that secreted product, as is typical of signaling ligands but can be true of other types of proteins as well.

For mosaic clones of mutations affecting genes with cell autonomous function, only cells in the mutant clone will have a mutant phenotype, whereas surrounding wildtype cells will appear normal. By contrast, for mosaic clones affecting genes encoding proteins with non-cell autonomous functions, wildtype cells surrounding the mutant clone will have a mutant phenotype. Moreover, the distance at which that nonautonomous function can exert influence tells us something about the nature of the gene product. In the case of proteins that exert their effects only on immediately adjacent cells, such as those effecting short-range signals among neighbors, the nonautonomous effect will probably be observable only in cells close to the mosaic clone. By contrast, for proteins with long-range effects, the radius of impact will be greater.

Beautiful Misdirection

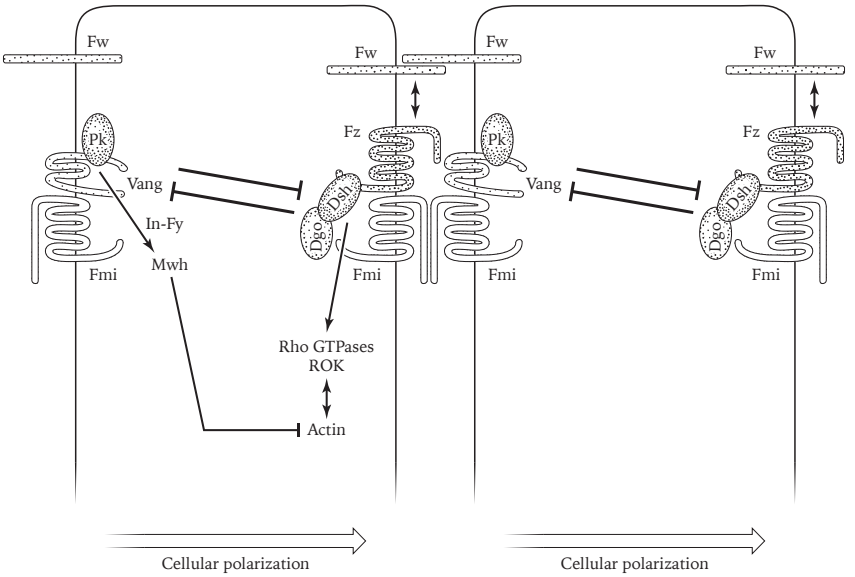
At least by the 1980s, researchers were using mosaic analysis in the fly wing to look at the effects on PCP of mutations in genes that would later be defined as components of a conserved PCP pathway (also known as the noncanonical Wnt pathway). D. Gubb and A. Garcia-Bellido used mosaic analysis to examine the effects of mutations in a gene called *frizzled* on polarity of bristles on the adult cuticle and of the wing hairs (Gubb and Garcia-Bellido 1982). Among other things, their results added a layer of understanding regarding the mechanisms of PCP. The data suggested that, rather than simply reflecting the organization or presence of surrounding structures, such as the wing margin or the wing veins, the orientations of the wing hairs reflect region-specific cues (Gubb and Garcia-Bellido 1982); that is, wing hair cells are able to respond to local cues in addition to reflecting the overall wing structure.

In the late 1990s, several other genes involved in PCP in the fly were identified and characterized, building a clearer picture of how things work at a molecular and cellular level. In 1998, J. Taylor, P. N. Adler, and colleagues reported identification of a gene they called *Van Gogh*. They found that production of *Van Gogh* (*Vang*) mutant clones in the wing resulted in

a whirling pattern of orientations of the wing hairs, reminiscent of the whirling lines typical of the eponymous artist's paintings (Taylor et al. 1998). In 1999, another core component of the PCP molecular pathway was uncovered, as two research groups reported identification of a gene called *flamingo* (*fmi*) by one group (Usui et al. 1999) *and starry night* (*stan*) by the other (Chae et al. 1999), the latter name another nod to the similarity between the whirling directions of the wing hairs observed in *fmi/stan* mosaic mutant wings and the painter's style.

In addition to parallels to fine art, the researchers noted striking similarities between the wing hair phenotypes associated with these newly identified mutations and the types of disruptions caused by mutations in the *frizzled* gene or mutations in another previously characterized gene, *prickle*. As might be expected, upon testing for genetic interactions among the mutant fly strains, researchers uncovered functional relationships among *frizzled, prickle, Vang,* and *fmi/stan,* placing them in a common pathway. Moreover, in observing the behavior of cells surrounding mutant clones of *Vang,* Taylor, Adler, and colleagues noted a "domineering" cell nonautonomy. The polarity within the mutant clone appeared to exert an exaggerated effect on the polarity of wing hairs of surrounding cells. In further analyzing this domineering nonautonomous phenotype, which was also associated with an opposite effect on polarity as compared with that observed for *frizzled* mutant clones, the researchers were able to use genetic analysis to determine that *Vang* is required for *frizzled* function, refining the relationships among the genes (Taylor et al. 1998). Researchers were observing that, although the more long-range proximal-to-distal axis defined by Wnt signaling in the wing was intact, disruption of PCP components in clones of cells led to a local reorganization or mistaken interpretation of that signal, causing the mutant cells, as well as those bordering the sinkhole of the mutant mosaic clone, to reorganize along what was essentially a new axis. This in turn resulted in the nonrandom but whirling and misaligned orientations of wing hairs observed in and around clusters of cells in which a gene encoding a component of the PCP system was disrupted (Eaton 1997; Strutt and Strutt 2005).

Through these and other studies, researchers were able to home in on how PCP works at the level of genes, their protein products, and cells. Wnt, Four-jointed (Fj), and Dachsous (Ds) act at a longer range, setting up the proximal-to-distal axis, and other factors act locally to define asymmetries within cells (Devenport 2016; Strutt and Strutt 2005; Fanto and McNeill



Interaction of components of PCP across a two-cell border in *Drosophila*. Fw, Furrowed protein; Fmi, Flamingo; Pk, Prickle; Fz, Frizzled; Vang, Van Gogh; Mwh, Multiple wing hairs; In, Inturned; Dgo, Diego; Dsh, Dishevelled; Fy, Fuzzy. Redrawn with permission from Y. Yang and M. Mlodzik (2015), "Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt)," *Annual Reviews of Cell and Developmental Biology* 31: 623–646, Figure 1. Copyright © 2015 by Annual Reviews, www.annualreviews.org.

2004; Simons and Mlodzik 2008). Combining genetic studies with other types of analysis, such as detection of the distribution of proteins within cells, we now have a good look at how PCP works in the fly wing and some other cell types. Moreover, in the advent of DNA sequencing, including availability of full-genome sequences, *Drosophila* researchers and others were able to match up sequences of *Drosophila* proteins to identify counterparts in the human or mouse genome; find them; and then ask whether these genes have similar functions at the level of biological or biochemical function, and whether the products of these orthologous genes are localized to similar subcellular compartments. For PCP, as for many other processes, researchers found that the parallels are extensive. What had once been an obscure phenomenon studied in the fly became recognized as common to many species and relevant to a wide range of structures and functions.

Learning where proteins are located within cells further helps us understand their functions. The Vang protein is embedded in the plasma membrane, with one surface facing outward and the other facing in. The Frizzled protein (Fz) is a Wnt ligand receptor that also spans the plasma membrane. Together with Fz, Vang responds to an extracellular signal, setting up a gradient across which the cells will orient themselves. Through reciprocal antagonistic activities, Vang and Fz keep each other at bay, such that Vang becomes limited to one side of the cell, and Fz to the other. This asymmetry is echoed by sets of "downstream" interacting proteins, including Prickle and Furrowed on one side, and Disheveled and Diego on the other. Fmi protein appears to be localized to both sides and binds adjacent cells together, through the interaction of the exterior-facing surface of Fmi proteins on one cell with that of Fmi proteins on the surface of the adjacent cell (Struhl, Casal, and Lawrence 2012; Lawrence, Casal, and Struhl 2004; Casal, Lawrence, and Struhl 2006). Communication of the asymmetrical organization set up by PCP components Vang, Fz, Dsh, and so on can then be used as a kind of template or compass within the cell, orienting intracellular changes at the level of mechanics and engineering. This includes changes in the cytoskeleton, such as via protein-modifying activity of proteins called Rho GTPases and the Rho-associated kinase, ROK, which eventually accounts for the construction, movement, and maintenance of a cilium, bristle, or hair appropriately placed at one side of the cell—in line with the original planar axis—and not at the other side.

One component in particular, the Mwh protein, is thought to directly affect the cytoskeletal building block actin, preventing individual actin proteins from being linked together to form a fiber (Strutt and Warrington 2008; Yan et al. 2008). The absence of Mwh in the opposite subcellular region allows for formation of actin fibers, such that structures are built in one region and not in the other. We have made a full connection, from linking how cells first sense the planar axis (via the Wnt ligand signal) to how they use that information to set up an asymmetry within the cell (via the mutually antagonistic activities of Vang and Fz), and how this informational asymmetry leads to asymmetry in the positions of physical structures, via changes to polymerization of actin or tubulin (Eaton 1997; Simons and Mlodzik 2008; Devenport 2016).

Genes encoding proteins highly related to most components that act in the PCP pathway in *Drosophila* are also found in mammalian genomes, and to the extent that they have been characterized—including in the mammalian

inner ear—many of the genes appear to have similar roles and relationships to one another, compared with control of PCP in the fly wing. In 2003, researchers reported that PCP in the inner ear is disrupted in "looptail" mutant mice, in which *Vangl2,* a mouse ortholog of fly *Vang,* is mutated; as well as in "spin cycle" and "crash" mutant mice, in which an ortholog of *fmi* is mutated (Montcouquiol et al. 2003; Curtin et al. 2003). Subsequent studies have further refined our understanding of the parallels, and differences, between PCP components in flies and vertebrates (Wallingford 2012). As is true for many genes conserved between less complex organisms and humans, for several PCP genes there are several human genes related to the single representative in the fly, the way a professional carpenter's toolbox might have several hammers but an amateur's toolbox just one. Underscoring the origin of our understanding of the genes, many of the human orthologs of *Drosophila* PCP genes were named for their counterparts in the fly. The human orthologs of *frizzled* are called *FZD1* through *FZD10*; of *disheveled* are *DVL1*, *DVL2*, *DVL3*, and *DVL1P1*; and of *prickle* are *PRICKLE1*, *PRICKLE2*, *PRICKLE3*, and *PRICKLE4*. The list could go on (Simons and Mlodzik 2008).

PCP on the Move

In addition to learning about PCP from a molecular view, we have also learned more about what tissues and processes require PCP. Among these are two processes that involve cell movement: the formation of cellular tubes and the process of healing a wound. Regarding the formation of tubes, in vertebrates such as the mouse, the formation of the brain and spinal cord during development depends on an early event called neural tube closure, in which a sheet of cells destined to become neuronal tissues curls up—similar to the way we might curl a piece of paper to make a cylinder—then fuses to form the neural tube. Z. Kibar and colleagues found that mutations in mouse or human relatives of PCP genes, including *Vang,* are associated with defects in formation of the neural tube, consistent with a role for PCP in this process (Kibar et al. 2011; Kibar et al. 2007; Kibar et al. 2001). PCP genes are also required for formation of the mammalian heart, which also involves formation of tubular structures (Henderson and Chaudhry 2011). A specific example is the association of mutations in the mouse Prickle1 gene with developmental heart defects, in addition to defects in the cochlea of the ear and other tissues (Gibbs et al. 2016). Wound

healing similarly involves the establishment of directional cues within a sheet of cells, in this case with cells orienting themselves relative to the wound site, then migrating toward the wound to close it. Cells from the same Prickle1 mutant mice described by B. C. Gibbs, C. W. Lo, and colleagues as having cardiac and cochlear defects also exhibit defects in the reorientation and movement necessary to close a wound (Gibbs et al. 2016).

These findings echo what has been uncovered in *Drosophila,* with a role for *fmi* in directed movement by neuronal cells (Organisti et al. 2015) and roles for *Drosophila* PCP genes in oriented cell movement (Munoz-Soriano, Belacortu, and Paricio 2012). Progress in each area—fly and mammalian PCP—is now spurred through conversation, through either the published literature or direct communication and collaboration among researchers with shared interests. The results obtained using each system are compared and contrasted. This makes it possible for researchers to work toward a general understanding of PCP, regardless of system. This also helps researchers tease apart how core PCP components connect with organism- and cell-specific factors in ways that make it possible for the same components to be involved in building different structures and orchestrating different activities, bringing about different results.

Cell Biology and Genetics

The story of PCP involves an intimate link between the signaling proteins that set up asymmetry across a sheet of cells and within them, and the cytoskeletal proteins that do the engineering work of building physical structures that reflect asymmetry. Genetic analyses pointed to some of the relationships between the information molecules and the structural ones, with genes encoding components of the cytoskeleton identified as modifiers of the PCP pathway or outcomes, such as when mutant clones of these cytoskeletal genes exhibit similar mutant phenotypes in the wing. The distinction between information and structure is not absolute. Fmi is thought to form "bridges" that are both informational and structural cues regarding polarity (Struhl, Casal, and Lawrence 2012). Studies in *Drosophila* are contributing in other ways to our understanding of cytoskeletal components, the extracellular matrix, and other structural proteins, and their relationships to signaling pathways, cellular activities, and developmental events. For example, studies of the *Drosophila* tracheal system, a branched system

of tubes that allows oxygen to reach internal tissues, are helping researchers uncover what extracellular proteins might be required for formation of tubes (Dong and Hayashi 2015); and studies in *Drosophila* of the gene *short stop,* which encodes a member of a family of large proteins called spectraplakins, are helping to uncover the roles of those proteins in cells (Hahn et al. 2016). With regards to PCP, whether the organization of structural proteins reflects or instructs PCP, or both, has remained an open question (Lawrence and Casal 2013).

From PCP in Flies to a Broader Understanding

For PCP, as for Hh, Hippo, and other pathways, discovery was spurred through genetic analyses in *Drosophila* and later extended to the study of vertebrates. We can appreciate now that there is a meaningful parallel to be drawn between defects in the fly wing and a mammalian epithelium despite the fact that these are very different tissues. By the late 1990s, molecular cloning of genes was beginning to reveal the extent of conservation between genes in flies and in the human genome. It was around this time that the study of PCP in vertebrates started to take off. The mammalian studies leveraged what had been learned about the genes, pathways, broader networks, and dynamics of PCP in *Drosophila* to help gain an understanding of tissues within our own bodies in which PCP is at play. As what goes right can go wrong, the understanding we have gained about PCP also affects our understanding of diverse human disorders and diseases, including hearing loss (Lu and Sipe 2016), diseases of the heart (Henderson and Chaudhry 2011), kidney (Carroll and Yu 2012; Fedeles and Gallagher 2013), and lungs (Yates and Dean 2011). The human Fz-related genes also provide an example of the fact that Nature does not just reuse but also co-opts. In human cells, the Fz-related receptors (FZDs) are exploited as a cellular inroad by *Clostridium difficile,* a pathogenic bacterium that can infect the colon, commonly leading to diarrhea and contributing to tens of thousands of deaths per year in United States alone (Tao et al. 2016; Lessa et al. 2015). Thus, studies that started with a curiosity—regarding how the hairs of the fly wing know to bend the same way—have impacted our understanding of a wide variety of biological processes, and provided hints regarding the relationships of PCP genes to disease.

Passing Notes to Your Neighbor

PCP explains how a tissue can set up axes and define asymmetric cellular structures along that axis. But we can take a step back and ask what tells a regularly-spaced subset of the cells in an epithelium to form sensory cells, whereas surrounding cells take on a supporting role. Before a sensory cell can extend a process in any direction, it must first take on the right identity. Cells in the epithelial sheet must be subdivided into the lucky few cells that will become decorated sensory cells, complete with wing hairs, bristles, cilia, or the like, and those that will take on the more modest role of support cells. Put another way, within an epithelium, different cells must adopt distinct cell fates, which in the case of the *Drosophila* wing epithelium comprises sensory and support cells organized in a distinct, regular, quilt-like pattern.

In some epithelial cell types, this prequel to PCP is controlled by a signaling pathway called the Notch pathway, so-named for the notched-wing phenotype of the flies that started researchers on a path to study of another conserved set of genes (Eddison, Le Roux, and Lewis 2000). Mutant flies with notches of tissue missing from the tips of their wings were first reported in 1914; shortly thereafter, notch-winged mutant flies were successfully bred in the lab and the gene was mapped to the fly X chromosome. Like *hedgehog* and PCP genes, the *Notch* gene is important for signaling, and indeed, is a member of another conserved signal transduction pathway. As in the definition and refinement of other pathways, researchers identified additional components of the Notch signaling pathway through identification of mutant flies with similar phenotypes or with genetic interactions with known members. Among the genes identified in the early years based on mutations that affect the wing and turn out to act in the same signaling pathway as *Notch* are the gene *Serrate* (first reported in 1939), mutations that cause the wings to be "irregularly notched at the tip," and two genes for which mutation results in thickening of the wing veins, *Delta* (1923) and *deltex* (1931) (Bridges and Brehme 1944).

With signaling ligands such as Hh, the message is sent via release by one cell of the Hh ligand, which then travels some number of cell distances away—a few cells or many—before that ligand is detected. Notch signaling, by contrast, can relay a message in a more discrete fashion. Delta, the Notch pathway signaling ligand, is not released by the signal-sending cell into the extracellular space; instead, it remains anchored in a membrane of the Delta-expressing cell. Thus, a Notch pathway signal only occurs when

a Delta-expressing cell is adjacent to a cell expressing the Notch receptor, such that ligand and receptor encounter one another, as two people might reach through a fence to shake hands. This distance-limited signaling is perfect for the kind of "lateral inhibition" necessary to establish a regular pattern of sensory cells, each surrounded by supporting cells, within the cochlear epithelium or the fly wing. Delta-expressing cells will take on one fate, Notch-expressing cells another, with their respective roles reinforced through transduction of the Notch signal in the receptor-expressing cells but not in others. Consistent with this idea, Notch signaling provides a way for one cell to tell surrounding cells to step out of the limelight and take on supporting roles, establishing the regular pattern of sensory cells peppered among the support cells in an epithelium (Eddison, Le Roux, and Lewis 2000).

For decades, the study of the Notch pathway has been a focus of intense study relevant to a wide range of topics and species, with the information learned enough to fill several books. Cells in various organisms take advantage of the mechanism for local communication provided by Notch to solve a number of problems. In flies, for example, *Notch* was recognized early on as a "neurogenic" or nerve tissue-inducing gene, and the Notch signaling pathway has since been found to be required for development of the human brain (Alberi et al. 2013). Notch signaling also has known relevance to an increasingly long list of human diseases, including Alzheimer's disease; inherited disorders such as Alagille syndrome and the associated heart defect known as tetralogy of Fallot (Grochowski, Loomes, and Spinner 2016); cancers of the lung, breast, kidney, ovaries, liver, skin, and brain; and some types of leukemia and lymphoma (Ntziachristos et al. 2014). The relationship between Notch signaling and cancer is complex. For example, whereas in some tissues, the turning off of Notch signaling is associated with the cancer state, for other tissues, it is not inactivation but hyperactivation of Notch signaling that is thought to lead to disease. For Notch-associated cancers, determining the status of Notch signaling in the cancerous cells—active or inactive, on or off—might help physicians predict outcomes and make informed decisions regarding appropriate therapeutic treatments; detection of specific proteins, sets of RNAs, or mutations can be used as indicators of Notch signaling pathway status (Takebe et al. 2015).

Theme and Variations

In addition to approaching problems from the perspective of a common cellular phenomenon—as in the case of PCP apparent in different types of epithelia—we can also approach problems from the perspective of equivalent organs (Appendix B). We can ask, for example, what about flies and hearing? Flies can both generate and detect sounds. As part of a stereotypical set of mating rituals, male flies sing a courtship song to females by vibrating their wings (Murthy 2010), suggesting the importance of hearing to continuance of the species. Insects detect sound via a substructure of the antenna called the Johnston's organ or JO, which is connected to the brain via neurons (Eberl and Boekhoff-Falk 2007). Much is known about how signals induced by sound are transmitted and interpreted by neurons in the *Drosophila* brain, including a role for TRP channel proteins (Chapter 8). Moreover, there are parallels between the genes and activities that govern development of the fly JO and the mammalian cochlea, as well as parallels regarding how sound signals are transmitted by neurons in the brain of a fly or a human (Albert and Gopfert 2015; Boekhoff-Falk 2005; Ishikawa and Kamikouchi 2015).

In one study, researchers identified close to 300 genes associated with the JO and found that, for 20 percent of them, there are related genes in humans that have been associated with hearing disorders (Senthilan et al. 2012). Another study revealed similarity at the molecular and functional levels in auditory organs in *Drosophila* and humans, specifically looking at the role of proteins called myosins, which are associated with an inherited form of deafness (Li, Giagtzoglou, et al. 2016). *Drosophila* is also being used in the study of noise-induced hearing loss (as is an even more unexpected animal, the sea anemone) (Christie and Eberl 2014; Christie et al. 2013). Exposure of flies to loud noises causes damage at the cellular level that parallels what is observed for noise-induced damage in humans, providing an assay that can be combined with the genetic approach to identify relevant genes.

Whether approached through common genes and cellular mechanisms, as for PCP, or through commonalities in overall function, as in studies of fly hearing, what is learned in *Drosophila* can lead biologists, including those with a specific interest in understanding human disease, in new and rewarding directions. C. Stern proposed in 1954 that "if we gain some knowledge as to the why and the where" a few specific fly bristles form—on

its own, a seemingly obscure topic, "we may throw light on the whole problem, not just on two or three bristles" (Stern 1954). In the case of PCP and Notch pathway components, the pleiotropic nature of the genes adds emphasis to the findings, as does the conservation of these genes among species that diverged long ago from a common ancestor. Similarly, the experimental approaches available for study of the fly—the genetic approach, mosaic analysis, and so on—are used iteratively, put to use in different contexts to accomplish related tasks. We apply the same experimental techniques to different topics, and allow the results to lead us in exciting new directions.

CHAPTER SIX

DIFFERENCE

MANY OF THE MUTANT FLY STRAINS IDENTIFIED IN THE EARLY YEARS OF fly research had mutant phenotypes so subtle it is a wonder the differences were noted at all. An extra speck of color at the base of the wing, slight differences in eye color, and small differences in the thickness or pattern of the veins on the wings are all among the differences spotted by early *Drosophila* researchers as they stared at scores of otherwise wildtype flies through the microscope. By contrast, a few mutant strains identified in the early years, and later, are shocking oddities. Researchers found flies with legs where the antennae would normally be; flies with a second pair of wings where the halteres should be; and flies with foreleg-type structures on their second or third pairs of legs. This class of mutant phenotype, in which one body part is substituted for another, had been noted previously in plants, and in the 1890s the phenomenon was termed "homeosis" by biologist W. Bateson (McGinnis 1994; Lewis 1994). In addition to these "homeotic" phenotypes, other types of oddities were also noted among collections of mutant flies, including flies in which a body part was essentially absent, as in *vestigial* mutant flies, which have mere stubs for wings, or *eyeless* mutant flies, which range in phenotype from small eyes to, as the name suggests, no eyes at all.

More than mere curiosities, mutant fly strains in which one body part is replaced with another or is missing turned out to be highly informative, helping us understand how the product of one gene can orchestrate the formation of an entire, discrete, complex structure like an antenna, a leg, or an eye. For one thing, the isolation of these flies suggested the presence of "master control genes" capable of directing the formation of an entire tissue, with all its component cell types. The later molecular characterization of these genes helped biologists get a handle on what types of genes—that is, what protein products and cellular activities—are capable of acting as master controllers. But the impact went further, bordering on the philosophical. Information gained from identifying the DNA sequence of homeotic genes and their positions in the genome, largely uncovered in the 1980s, led to findings relevant to multicellular organisms as diverse as sea urchins and humans: findings so surprising that "not a single biologist ever anticipated" the deep connections among organisms that they revealed (Carroll 2005).

Antennae versus Legs

Antennapedia (*Antp*) mutant flies have legs growing out of their heads. Images of these flies have appeared in many science magazines and textbooks, and were even included in an episode of a sci-fi television show. From the viewpoint of a biologist, the isolation of a mutation in which one body part is turned into another suggests something about how our various body parts form. Specifically, mutations like *Antp* suggest the presence of a master control gene capable of directing the path down which some set of cells in the embryo or larva travels, turning on and off the right sets of other genes in order to make a specific body part in the right region of the body. Like other mutant flies, flies with mutations in the *Antp* gene or other homeotic genes were subjected to the usual tests available to twentieth-century biologists: they were crossed to one another to determine which mutations were in the same gene and which affected different genes, and the corresponding genes were mapped to specific regions of the chromosomes. Intriguingly, several of the homeotic mutations mapped to one of just two specific regions on the right arm of chromosome three—an early hint of something unusual regarding organization of these genes along chromosomes.

When the sequence of the *Antp* gene and surrounding gene regions was identified in the early 1980s, researchers were able to get a first look at what the amino acid sequence of the Antp protein might be (Scott and Weiner

1984; McGinnis, Levine, et al. 1984; McGinnis, Garber, et al. 1984). A researcher staring at a newly identified protein sequence often hopes that the sequence will be similar to a protein of known biochemical function—an enzyme, say, or a receptor. When it does, we can match up what was learned from the mutant phenotype with the biochemical function revealed by the sequence and begin to gain a sense of both what the protein does and how it does it. The Antp amino acid sequence looked largely unlike the sequence of most other proteins identified at that time. But a hint of its biochemical function came from a region that had some similarity to a protein found in yeast that was known to function as a DNA-binding transcription factor—a protein capable of turning on or off specific set of genes. Using this insight, researchers were able to ask whether Antp was also a transcription factor, and showed that yes, it is. The finding makes sense. What kind of gene can be a master control gene? One that encodes something that turns on or off a specific subset of genes, such as genes required to make a leg or an antenna, or to suppress formation of another structure. Consistent with this, researchers found that the *Antp* gene is normally expressed in a specific subregion of the fly; there is a direct relationship between where *Antp* is expressed—such that the Antp protein is made in the cells—and where specific structures form.

The region of the Antp protein that binds DNA was named the "homeobox" domain (after "homeotic"), later shortened to Hox (McGinnis, Garber, et al. 1984). Using the techniques of the day, which included the use of radioactively labeled DNA from a Hox gene in one species to identify pieces of DNA encoding other Hox proteins in the same species (a test known as a "blot") or in different species (a test known as a "zoo blot"), researchers identified more and more Hox proteins and continued studies of their functions. Many *Drosophila* homeotic genes turned out to encode Hox proteins. Hox family proteins can be further subdivided into subgroups, with some Hox proteins sharing amino acid sequence similarity only in the Hox domain itself and others having similarity in additional regions as well. Researchers now recognize 16 different subcategories of Hox proteins, found in various organisms (Burglin and Affolter 2016). But one thing remained clear. What most or all Hox proteins appear to have in common is the ability to bind DNA along chromosomes and influence transcription of genes near those binding sites.

Identification of master control genes like *Antp* was quite an advance, as was the recognition that Hox protein-encoding genes are common in the

genomes of multicellular animals like the fly, the mouse, or humans. The story might have ended there. And it would have been an impactful one, a "first in fly" finding memorable for being the first identification of a Hox protein. But the *Antp* story has an added layer of complexity and interest. Learning about Antp did not just teach us about master control genes and identify a large family of transcription factors. There was one more surprise in store, one that helped launch a new field of study, evolutionary developmental biology or "evo-devo."

To learn the surprise, we have to return to genetic mapping and to some of the other homeotic genes.

When Location Reflects Function

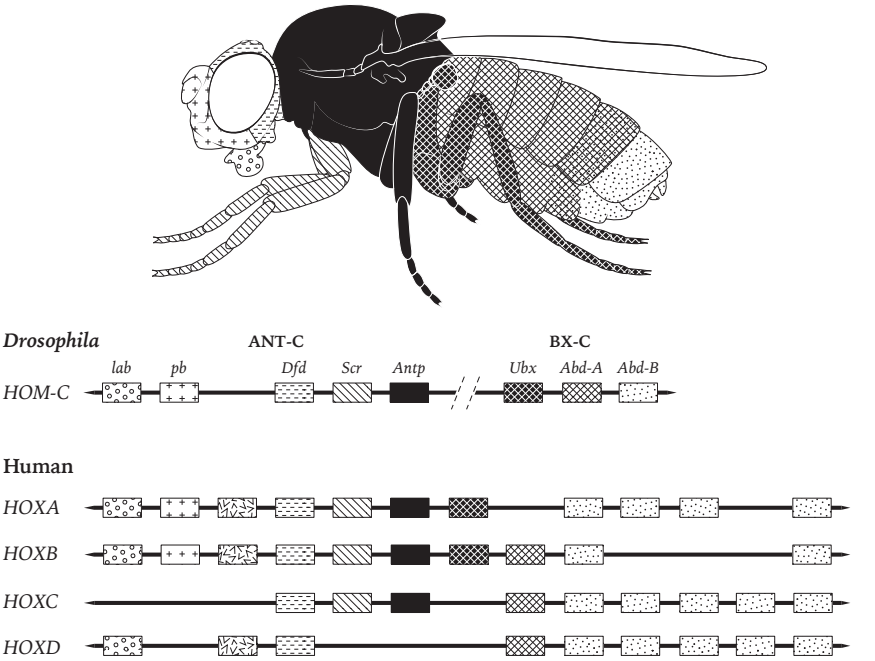
In eukaryotes, most genes appear to be randomly distributed in the genome. There is no obvious relationship between their positions in the genome and their functions or the locations of their resulting gene products within the cell. For example, although the *white* gene is located on the X chromosome, many other genes required for the ordered enzymatic activities that make eye pigments are nowhere near *white,* with *vermillion,* for example, far from *white* on the X chromosome; *cinnabar* on the second chromosome; and *scarlet* on the third. We can extend this further to patterns of expression of genes. When researchers used radioactive probes or color-precipitating dye technologies to ask in what subset of tissues a given gene is expressed (that is, where its RNA can be detected), we can find genes that are off early in embryogenesis and turned on later; genes that are expressed in the eye but not the wing, or in the eye and the wing but not the gut. The list goes on and on, including genes expressed in all cells or only in a very limited set of cells, at all stages or at limited stages, in large swaths of the embryo or in discrete stripes, corresponding to the segments. But these patterns have no obvious relationship to the locations of the corresponding genes in the genome—different genes expressed in the same striped patterns can be found in various regions of the genome; genes expressed in only the eye or wing likewise can be found in various places along chromosomes; and genes expressed in all cells can be next door to genes expressed only in very limited cell types.

Hox genes turn out to provide an exception to this rule. An early hint that something unique was going on was the recognition that the *Antp, fushi tarazu,* and *Ultrabithorax* genes are near one another on chromosome three

and encode similar proteins, including a stretch of sequence in which fifty-one of the sixty-three amino acids encoded by the genes are identical (Scott and Weiner 1984; McGinnis, Garber, et al. 1984). Researchers eventually worked out that *Antp* and several other Hox genes are found in chromosomal complexes or gene clusters, as reflected first in classical mapping data, with several genes of apparently related functions mapping within a close distance of one another; then in polytene chromosome mapping information, where again, the close proximity of the genes was evident; and finally, as revealed by DNA sequence data. Altogether, the genes were revealed to participate in common functions, encode similar proteins, and sit together in a defined order in two chromosomal regions. The region that includes *Antp* became known as the Antp Complex (ANT-C) and the other, the Bithorax Complex (BX-C).

It was not just clustering of the genes in general that was striking. The roles of the genes in the organism—their respective requirements in anterior, middle, or more posterior regions, as reflected in the subset of cells in which specific subsets of Hox genes are expressed—turned out to be reflected in their physical organization along chromosomes. Genes expressed at the anterior end of the fly are at one end of the cluster, those expressed in the middle are more central in the cluster, and so on, with the position of the gene within the cluster—at the level of the DNA itself—sharing a direct relationship with requirement of the genes to direct formation of specific subregions along the anterior-to-posterior axis of the fly.

Perhaps the biggest surprise came in exploring the organization of Hox genes along the chromosomes of other organisms. Researchers found that the organization true in flies is also true of that of Hox genes in the genomes of other animals, including the human genome. Mammals like us have Hox genes organized in Hox clusters. Moreover, the patterns of expression and functions of the Hox genes in a developing mouse, say, similarly function as master control genes and reflect the positions of the genes within the clusters, as was shown by H. LeMouellic, Y. Lallemand, and P. Brulet in 1992 by replacing a mouse Hox protein-coding sequence encoding an enzyme whose pattern of expression can be monitored using a colorimetric test (Le Mouellic, Lallemand, and Brulet 1992). There is no obvious mechanistic reason why this subset of genes would be organized this way—many other genes expressed in similar, coordinated patterns are scattered about in the genome. In addition, huge spans of time separate our evolution from the common ancestor in which a Hox gene complex arose.



Hox gene expression domains and gene order. Top, regions of expression of Hox genes in the adult fly and the positions of the corresponding genes on chromosome three. As indicated by shading, the order of the genes along the chromosome is reflected in the expression patterns of the genes along the anterior-posterior axis. Bottom, human Hox genes are also organized in clusters, with orthologs found in roughly the same order as in the fly. Gene order in mammalian Hox clusters also reflects the organization of the body plan.

Nevertheless, the clustered organization of Hox genes has been preserved over time and is fundamental to how we develop, with all the right parts in all the right places. Many years after human Hox genes were identified, researchers found evidence that mutations in human Hox protein-coding genes can result in homeotic-type transformations. In 2012, Spielmann, Mundlos, and colleagues reported that mutations in *PITX1*, which encodes a paired-type Hox protein, are responsible for an inherited disorder called Liebenberg syndrome, which is characterized by the formation of leg- and foot-like bone structures in the arms and hands (Spielmann et al. 2012). Other human Hox genes are associated with other types of disruptions of

the body plan, including polydactyly and other abnormalities of the fingers or toes (Quinonez and Innis 2014).

The finding that Hox genes are organized in clusters, that the clusters reflect the pattern of the organism, and that the clustered organization has been so preserved over time, sent shockwaves through the field of biology, changing our views of development and evolution, and helping launch the evo-devo research field, which combines genomic analyses and embryological studies. More broadly, this new understanding of Hox gene conservation revealed a profound connection among organisms as different in outward appearance as a fly and a human being, or a snake and an elephant. Despite the millions of years that separate us, we share something fundamental with regard to how we form and where that form has its origins.

Flies without Eyes—the EYA Transcriptional Network

True homeotic mutations like the *Antp* mutation result in the transformation of one structure into another. But in addition to homeotic mutant flies, researchers isolated, early on, other mutant flies that similarly hinted at the presence of master control genes: mutant flies in which one structure, such as the eye or the wing, was not replaced with another but instead was much reduced in size or simply absent. An example is the *eyeless* (*ey*) mutant flies. The first *ey* mutant fly strain, in which the fly's eyes were reduced to about half their normal size, was reported by M. A. Hoge in a paper with the modest title "Another gene in the fourth chromosome of Drosophila," which appeared in *American Naturalist* in 1915 (Hoge 1915). The title of the report probably reflects the emphasis of the times on genetic mapping of mutations, rather than on characterizing the resulting phenotypes. In the paper, Hoge presents the number of flies of various phenotypic classes observed after setting up genetic crosses designed to figure out on what chromosome the mutant gene is likely to reside. Hoge initially presents evidence that the *eyeless* mutation is not linked to the X, second or third chromosome, suggesting that the mutation is on the tiny fourth chromosome. She then provides further evidence that *ey* is indeed on chromosome four, leading to the conclusion that is reflected in the title of the paper.

Additional mutant alleles of *ey* were identified subsequent to this initial report. These include a second mutant strain in which eye size was reduced

even farther, averaging 25–50 percent of the normal size of the eye, and a third *ey* mutation noted as appearing spontaneously in a fly stock from Sweden (Bridges and Brehme 1944). In the hundred or so years that followed the initial report, about forty different mutations in *ey* have been isolated and reported in the literature; altogether, about eighty mutant fly stocks related to the *ey* gene are available from public stock centers. In addition to new mutant alleles of *ey,* researchers also found flies with mutations that map to different chromosomes but are in a similar reduced or absent eye phenotype, notably *twin of eyeless* (*toy*), *eyes absent* (*eya*), and *sine oculis* (*so*), which is Latin for "without eyes."

Similar to *Antp* mutations, the mere existence of mutations in single genes that could lead to the absence of an entire structure suggested to researchers that some genes function as master control genes able to send the subset of cells that express the gene down a path toward formation of a given structure, in this case the eye. Definitive evidence that *ey* encodes a master control gene came in 1995, when W. J. Gehring and colleagues reported that when they forced cells to express *ey* in tissues that do not normally express the gene, they observed something remarkable: ectopic expression of *ey* in tissues such as the antennae, legs, or wings led to formation of clumps or outgrowths of tissue that had the attributes of a fly eye, complete with red-pigmented facets organized into the crystalline lattice characteristic of a normal fly eye (Halder, Callaerts, and Gehring 1995). Despite the large span of evolutionary time that separates flies and humans, as well as the obvious differences between the compound eye of the fly and the human eye, Gehring and colleagues found that the *ey* gene had relatives in mammals that appeared to have similar functions. A gene (later named *Pax6*) associated with a "Small eye" mutant mouse strain turned out to be related to *ey,* and a human gene associated with congenital aniridia, or the absence of an iris, are orthologs of *ey* (Quiring et al. 1994). Indeed, Gehring and colleagues found that the conservation of function has been so preserved among these species that like expression of *ey* itself, expression of the mouse *Pax6* gene in *Drosophila* was sufficient to induce formation of eyelike structures (Halder, Callaerts, and Gehring 1995).

The protein product of the *ey* gene, as well as those of the *toy* and *so* genes, is a transcription factor. Specifically, the Ey protein is a member of the "paired box" (PAX) subfamily of Hox proteins. If we imagine that DNA sequences bound by the Ey transcription factor are present near the subset of genes required for eye formation, we can begin to view, at least in

simple terms, how Ey functions as a master controller. The set of genes with Ey binding sites in their promoter regions will be "on" in cells in which Ey is present and "off" in other cells. The true picture, however, turns out to be more complex. The *ey* gene forms part of a network, together with *toy, eya, so,* and a gene called *dachshund* (*dach*), whose protein products control one another's gene activity in addition to regulating the set of genes that define the eye. The specific organization of this interdependent transcriptional network, referred to as the EYA network in humans, turns out to be similar in mammals (Wawersik and Maas 2000; Donner and Maas 2004). That is, orthologs of the *Drosophila* Ey, Toy, Eya, So, and Dach proteins not only are encoded by the human genome and similar to their fly equivalents in their protein sequence and function, but furthermore have similar relationships with one another and share an involvement in eye formation. Consistent with this, disruptions in human EYA network genes are associated with genetic disorders of the eye, including aniridia (as in disruption of *PAX6*) and ocular coloboma, which is characterized by small size or absence of various eye structures (Azuma et al. 2003; Donner and Maas 2004).

For *ey,* as in other examples, what was learned in the fly led to insights into what human genes are involved in a process (in this case, eye development), what they do, and how they might be wired into networks. Results obtained using the simpler system facilitated study and informed our understanding of more complex animals. Researchers continue to ponder the meaning of the commonality among organisms regarding how the body plan is organized and specific structures defined along its length, as well as how seemingly small differences in gene organization or DNA sequence can lead to profound differences in the body plan, such as the formation of a long and many-legged creature, as in a centipede (Hughes and Kaufman 2002), or a long and limbless creature, as in a snake (Di-Poi et al. 2010). Nobel laureates E. Wieschaus and C. Nüsslein-Volhard have commented that this "deep evolutionary conservation of molecular mechanisms of development had not been expected" (Wieschaus and Nüsslein-Volhard 2014) and that "The discovery of the homeobox . . . enforced the idea that evolutionarily distant organisms might share common developmental pathways and common genetic circuits. This idea is now taken for granted. . . . The discovery of the homeobox provided one of the best and most convincing examples of that homology and probably more than any other single observation transformed thinking in the field" (Wieschaus and Nüsslein-Volhard 2014).

Rear Legs Become Front

A different set of homeotic mutant phenotypes helped researchers uncover another, separate system for turning genes off and on, one in which the goal of transcription regulation is accomplished in a very different way as compared with binding of discrete sequences by transcription factors. The front legs of a male *Drosophila* have a feature called the sex comb, a dense row of bristles, located on the fly equivalent of the shin. The sex combs serve as a landmark that (in males) clearly distinguishes the first pair of legs from the second and third pairs. In the 1940s–1960s, mutations in three genes that cause a homeotic transformation of the second and third pairs of legs into foreleg-type legs were identified. Based on the phenotypes observed in the mutant fly strains, the genes were named *Polycomb* (*Pc*), *extra sex combs* (*esc*), and *Multiple sex comb* (*Msc*), which has the official FlyBase name *Sex combs reduced* (*Scr*) (Tokunaga 1966; Hannah-Alava 1958; Lewis 1947; 1956; Puro and Nygren 1975). C. Tokunaga described the phenotype of the *Msc* mutant fly strain as characterized by a "differentiation of transverse bristle rows between the longitudinal rows 6 and 8 on each basitarsus [base of the toe] and the distal part of the tibia [lower leg]" (Tokunaga 1966). For these mutant flies, as well as similar mutant flies such as *Polycomblike* (Duncan 1982), homeotic transformation of rear legs to front-leg identity made the mutant strains intriguing to researchers. These fly strains were extensively studied in the latter half of the twentieth century by several researchers, including A. Hannah-Alava, I. M. Duncan, and P. Lewis, as well as E. B. Lewis, who would later share the Nobel prize with Nüsslein-Volhard and Wieschaus (Hannah-Alava 1958; 1969; Lewis 1978; 1982; Duncan 1982).

Using genetic tests to ask whether two mutations are in the same or different genes usually results in straightforward, unambiguous answers. For recessive genes, if two different mutations affect the same gene, we can make predictions as to how they will perform in two genetic tests. First, if the mutations affect the same gene, genetic mapping will place the genes in the same region of the same chromosome. Second, if two mutations affect the same gene, when flies with one mutation are crossed with flies carrying the other, the resulting offspring will almost always display a mutant phenotype, since no normal copy of the gene is present. However, rather than providing straightforward results in these tests, the relationship among mutations that we now know affect distinct genes was ambiguous

as late as the 1970s. In particular, this was true for a subset of mutant fly strains with *Pc*-like phenotypes for which mapping experiments placed them, like *Pc,* on chromosome three. Since *Pc* mutations can have both a dominant phenotype (ectopic sex combs) and a recessive phenotype (lethality), the predicted outcomes of the tests were complex. Nevertheless, standard tests could be applied, and would typically have provided unambiguous results. What researchers found, however, was that for *Pc* and *Msc,* as well as for a mutant strain called *Sex combs extra* (*Scx*), the results of these tests were ambiguous. Genetic mapping, as well as information gained from analysis of polytene chromosomes, revealed that all three mutations map to a common subregion of chromosome three. Recombination between *Pc* and *Msc* was rare, with a map distance of just 0.3 cM between them (Hannah-Alava 1969: Puro and Nygren 1975). Taken alone, these data would suggest that the mutations affect the same gene. But the results of the complementation test told a different story. In this test, the three mutations behave in a manner that suggests they affect three different genes. Flies with one mutant copy of *Pc* and one mutant copy of *Scx* or *Msc* survived, whereas flies in which both the maternal and paternal copies of *Pc, Scx,* or *Msc* are mutant were unable to survive (Puro and Nygren 1975).

Researchers crossed and counted more and more flies, and eventually determined that the mutations are in three different (albeit nearby) genes, with *Pc* and *Msc* representing separate genes that became known by those names, and *Scx* a mutant allele of *Antp*. Though distinct, the genes are functionally interrelated. We now know that the protein products of these genes physically interact with one another, and also with the chromosomal region that encodes them (as well as other regions of the DNA). This, too, was in part revealed by genetic tests. For example, in the absence of *Pc* activity in cells of the central nervous system, the pattern of expression of genes in the *Antp* gene region, as well as in the *bithorax* region, is "dramatically altered" (Wedeen, Harding, and Levine 1986). Through detailed genetic analyses, researchers discovered that *Pc* affects the levels of expression of other genes—it acts as a regulator of the expression of other genes. Specifically, evidence suggested that the normal activity of the Pc protein is to repress the expression of other genes, which become held in an off state when Pc is present. Moreover, researchers found that mutations in *Pc* can result in phenotypes that affect in flies at a variety of lifecycle stages and tissues (Lewis 1978; Haynie 1983; Denell and Frederick 1983), suggesting that the *Pc* gene is highly pleiotropic.

Already by the 1980s, *Pc* was considered "one of the most extensively analyzed examples of a regulatory gene" (Wedeen, Harding, and Levine 1986). Eventually, molecular cloning and characterization of Pc and related proteins revealed the molecular function of two large multiprotein complexes in which Pc becomes incorporated, the "polycomb repressive complexes," PRC1 and PCR2. Moreover, functional conservation of Pc activity—and more broadly, the composition and function of PRC1 and PCR2—turned out to extend not only to vertebrates, including humans, but also to fungi and plants, in which PRCs similarly have repressive activity (Mozgova and Hennig 2015). In *Drosophila,* PRC1 comprises the protein products of the genes *Pc, Polyhomeotic-distal, Polyhomeotic proximal, Posterior sex combs, Suppressor of zeste 2,* and *Scx* (orthologs of these genes in the human genome are *CBX2,4,6,7*, and *8*; *PHC1,2*, and *3*; *PCGF1,2,3,4,5*, and *6*; and *RING1A* and *RING1B*). PRC2 comprises the protein products of *Enhancer of zeste, S(z)12, esc, esc-like,* and *Caf1–55* (*EZH1* and *EZH2*; *SUZ12*; *EED*; and *RBBP4* and *RBBP7*).

To understand how PRCs repress gene expression, we must take a step back and think about "chromatin," DNA in the highly organized and structured state it takes on in the form of chromosomes within the nucleus of a living cell. As compared with purified DNA extracted from cells in a lab, which can be extended as long threads from the fine plastic point-tip of a pipette, DNA in the form of chromatin takes on a very regular structure. Importantly, this structure has a relationship to what genes are expressed in a given cell, with regions buried deep in the three-dimensional looping of chromatin less accessible to the transcriptional machinery, such that the genes are less likely to be transcribed into RNA. The PRCs, along with Trithorax Group (TrxG) protein complexes, which have opposing activity compared with the PRCs, are the "major opposing systems of epigenetic regulation" in plants and animals (Mozgova and Hennig 2015).

Careful Control

What is "epigenetic" regulation? Or put another way, how does regulation by PRCs differ from regulation of gene expression by Hox proteins or other transcription factors? The differences can be likened to the control of traffic patterns. Transcription factors can be thought of as the stoplight system. Transcription factors bind specific regions of DNA nearby genes, giving a green or red light to the transcriptional machinery. The light might

be red or green at any given moment, but either way, the message conveyed is specific to one intersection. By contrast, epigenetic control by PRCs is more like the placing of traffic cones or blockades along lanes of traffic, restricting access. PRCs put marks on the DNA which, like traffic cones on a street, can be placed along any stretch of the DNA, can be moved around based on need, and can cordon off short or long distances, affecting access by the transcriptional machinery to one or many genes. Just as we rely on both a fixed traffic light system and a more flexible system of temporary road closures to direct safe and organized travel on roads, so, too, regulation by transcription factors and PRCs together accomplish the goal of carefully but not inflexibly regulating which genes are turned off and which are turned on (and at what levels) in each cell.

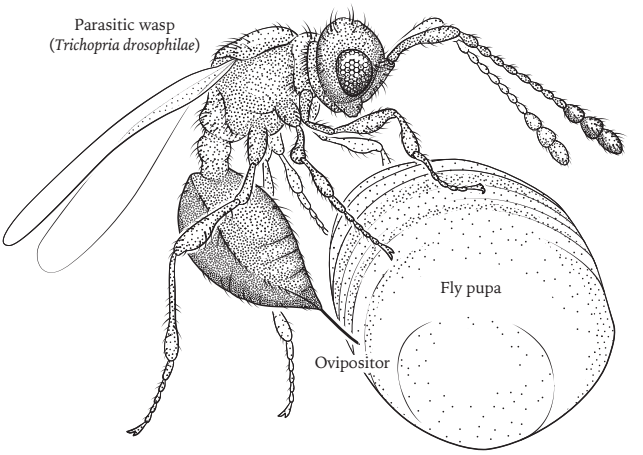
Why are living systems so paranoid, with multiple genes and mechanisms exerting control over the expression of genes? We can keep in mind that all the chromosomes, and thus all the genes, are present in the nucleus of each cell. As a result, each cell contains all the information needed not only to make the cell type it is meant to be, but also to generate every other cell type, including instructing a cell to carry out processes associated with development and final adult states. For a human, this means that our liver cells have all the information needed to build a brain; our skin cells have the information needed to build tooth or bone; and so on. For social colony-forming organisms such as bees or ants, this further means that each cell of a single individual in the colony contains the information required to make a queen, a drone, or a worker. With this in mind, it becomes easier to grasp why genes along the genome are subject to such exquisite control. Highly regulated transcriptional control, as in the EYA network, along with the very different type of control exerted by PRCs, together protect us against the deadly confusion that can result from turning a gene off or on at an inappropriate time or place within the whole.

CHAPTER SEVEN

DEFENSES

OUT IN THE WILDS OF A VINEYARD, ORCHARD, OR CIDER MILL, FRUIT flies face any number of predators: many types of bats, birds, frogs, insects, lizards, rodents, and snakes will happily snatch them up for a snack. Wild flies are also exposed to microbes such as bacteria or viruses that can infect and in some cases overtake them, and to larger, more sinister-seeming foes. Some species of parasitic wasp use their syringe-like "ovipositors" to inject eggs into fly larvae or pupae. The larvae then hatch and eventually kill the fly host, as they feed from inside and break through to emerge as adults. Similarly, some nematode worms partner with toxic bacteria to make a feast of fly larvae, boring into larvae and consuming them from the inside out. Flies are prone to vampiric foes also. Mites, small arthropods in the subclass of arachnids called Acari that also includes ticks, can make the life of a fly difficult, traveling on adults like unwanted baggage and threatening flies at their most vulnerable stages, as immobile embryos or pupae. To see a fly with a mite clinging to it evokes a pang of emotion; imagine a person carrying around a tick the size of a dinner plate. Not fun.

In contrast to wild flies, *Drosophila* cultured in a lab appear to lead a relatively secluded and protected life, interacting with few other species and free from the threat of large predators, unless of course the occasional re-



A parasitic wasp depositing eggs into a *Drosophila* pupa. The wasp uses its syringe-like "ovipositor" to inject fertilized eggs into the host. The wasp larvae will hatch inside the pupa and use it as a food source.

searcher armed with a pair of tweezers and a dissecting needle counts as a "predator." A vial of flies is not, however, a single-species monoculture. Like other animals, lab flies play host to a "microbiome" or set of beneficial bacteria, such as the set of bacteria normally present in a fly's gut. Moreover, despite the containment of lab flies inside the confines of a glass or plastic vial in an incubator that sits in a building, the outside world can still reach through and affect them. Harmful bacteria, molds, viruses, and mites occasionally make their way into lab fly stocks, threatening the hundreds, sometimes thousands of different genotypes of *Drosophila* kept in the lab as living research tools.

Of course, *Drosophila* are not helpless in their own defense, either in the wild or in the lab. Faced with threats, flies do as we do: they fight back—at a cellular level, at least. The ways in which flies fight off infection and other threats, at the level of genes and interconnected gene networks and pathways, turn out to be another fascinating set of examples of how study of the fly can provide new information that serves to further our understanding of ourselves. By providing testable hypotheses for gene and pathway function in our own cells, work in this area has accelerated the pace of understanding how a wide variety of organisms fight infection, rallying the troops to fight off would-be invaders. Studies using *Drosophila*

have also provided insights into how insects resist our efforts to kill them and how we defend ourselves against another threat—one that lurks within our genomes. When we consider the impact of immunity on human health, and remember that the fly is a close cousin of disease-carrying Dipterans such as mosquitoes, these areas of research take on a deep sense of importance and urgency.

If You Prick Us, Do We Not Bleed?

To study responses to infection in *Drosophila,* we first need a method for infecting flies in a controlled manner, with responses measured and documented over repeated trials, so that any divergence from the normal response—such as might occur in a specific mutant fly compared with wildtype—will be noticed. We need an assay. As it turns out, infecting an insect with a microbial pathogen is not difficult. One straightforward method is to take a pin, dip it into a culture of bacteria or fungus, and pierce an adult fly with the pin, giving the microbe access to the vulnerable soft interior normally protected by the hardened outside. Another approach is to feed microbes to flies, allowing them to colonize the fly gut, as has been done to facilitate the study of pathogens related to human digestive and lung disorders (Panayidou, Ioannidou, and Apidianakis 2014).

In response to an insult like a prick from a tainted pin, the insect's body reacts quickly. At the site of the wound, one type of blood-like cell facilitates formation of a clot, which in a fly takes the form of a hardened clump of "melanized" or darkly pigmented cell material. The clot is presumed to help cordon off and contain the would-be invasion, keeping it from spreading throughout the body. Farther away, in an organ called the "fat body," specialized cells launch an arsenal of weapons, up-regulating genes that encode short proteins called antimicrobial peptides (AMPs). Once produced, AMPs are released into the extracellular space, and into the "hemolymph" or blood of the fly. When the AMPs encounter a microbial foe, they can slip inside and weaken or kill it, or otherwise limit the invader's ability to make more of itself. Although they are not fully customized the way human antibodies are, targeting specific invaders, the different types of AMPs are specialized for attacks by bacteria, fungi, or viruses. Put another way, whereas vertebrates have an "acquired immune response" in which antibodies are custom-engineered in response to specific invasions (or mock

invasions in the case of vaccines), insects and many other animals are said to have only an "innate" immune response involving AMPs and other preprogrammed responses to general classes of invaders.

Here, the simpler system, the fly, presents a limit. One cannot use the genetic approach, or any of the other powerful experimental approaches available for a research organism, if that organism does not perform the biological task of interest. However, like flies, humans have innate immune responses; we have preserved this more ancient system and layered upon it the acquired immune system. Moreover, what is learned about innate immunity in *Drosophila* is relevant to understanding immunity in insect disease vectors, with the research in flies providing insights into how disease pathogens evade detection in mosquitoes and other vectors. Starting first with studies in larger insects such as grasshoppers and silk moths—a system studied at times by Louis Pasteur—then moving to the smaller *Drosophila* system, researchers have been able to piece together a fairly complete picture of what happens, at the level of genes and cells, when a fly's cells, or ours, face a threat and mount an innate immune response.

By the early twentieth century, it was known that insects' responses to infection include both local cellular responses and a more general response by the humoral system (Ferrandon et al. 2007). Eventually, researchers were able to purify, isolate, and determine the protein sequence of AMPs made by insects that had been infected, as well as demonstrate that these peptides can kill bacteria, fungi, or both. Like familiar antimicrobial drugs such as penicillin, these microbe-killing AMPs were given names with "-in" endings, such as "cecropin" and "diptericin." By the end of the twentieth century, and before the era of genome sequencing, researchers had already isolated more than 150 different AMPs from various grasshoppers, moths, and other insects. Owing at least in part to improvements in mass spectrometry technology, a method used to identify the amino acid sequence of a purified protein, by the 1980s researchers had isolated AMPs from *Drosophila* as well, even though extracting proteins from flies yields smaller samples compared with extracting proteins from much larger grasshoppers or moths.

Despite progress identifying AMPs, however, a mystery remained. The researchers had found that the insects mount responses in the form of AMPs. But how do insects know they have been invaded? And what turns on AMP production once cells detect an invasion? Moreover, how does an

organism induce a response not just locally, in the cells that have direct contact with the microbe, but also in cells far away from the infection itself? And furthermore, how do the cells know what type of microbe has invaded, so that they can mount an appropriate response?

Detect, Relay, Respond

Prior to the Heidelberg screen (Chapter 3), Nüsslein-Volhard and colleagues described a dominant mutation in the gene *Toll* that resulted in larvae lacking dorsal structures; the larval cuticles are "ventralized" (Anderson, Jürgens, and Nüsslein-Volhard 1985). By 1988, K. V. Anderson and colleagues had succeeded in sequencing a DNA fragment that corresponds to the *Toll* gene, and deduced, based on the predicted amino acid sequence, that the Toll protein is a membrane-spanning protein. The *Toll* mutant phenotype was shared by other mutations, suggesting roles for additional players in a shared pathway. Through work by many, a "Toll signal transduction pathway" involved in specification of the dorsal-ventral axes was identified. Information flow in Toll pathway specification of the dorsal-ventral axis requires the processing of an extracellular protein ligand, encoded by the gene *spätzle* (named for the noodle-like shape of *spätzle* mutant larval cuticles), followed by activation of the Toll receptor by this processed form of Spätzle protein and transduction of the signal inside the cell via intracellular effector proteins that fly researchers named Tube, Pelle, Cactus, and Dorsal.

The story might have ended there, with identification of yet another pathway involved in development, adding to our understanding of how signals set up the major body axes of the larva. However, when J. A. Hoffmann and colleagues went looking for genes that can signal cells to start making AMPs, they found something somewhat surprising; they found *Toll*. This receptor, originally identified based on its role in larval cuticle patterning, turned out also to link the external world of microbial invasion at the cell surface, as detected via specific proteins or other molecules present in or on the bacteria, fungi, or viruses, with the internal, intracellular world of the cell, initiating a response to such an invasion. When a microbe is present, the membrane-spanning Toll protein receives the "We're invaded" signal on the outside of the cell and conveys a "Take up arms" message within, leading to a cascade of events, including the binding of transcription factors to

specific DNA regions on chromosomes, up-regulating AMPs and other relevant genes. The finding earned Hoffmann a Nobel Prize.

Toll is another prime example of a gene that is both highly conserved and pleiotropic—presenting a single solution to a problem that gets applied in different cells to accomplish different goals. By the time the connection between innate immune responses and Toll was made, many of components of the Toll signaling pathway and their functional relationships to one another had already been characterized, through study of larval patterning genes. This provided researchers interested in the innate immune system with immediately testable hypotheses regarding what additional genes might be involved in the steps between activation of Toll and the turning on of an AMP gene. They looked for and found similarities between Tube, Pelle, Cactus, and other *Drosophila* proteins and proteins involved in immunity in mammals. Researchers compared the Toll signaling pathway with the mammalian immune surveillance system and found that the wiring up of the components that transduce the Toll signal during development and those that transduce the response to a pathogen in mammals turn out to be essentially the same, even though both the initiating message and the induced responses are different in the development and immune response scenarios (Ferrandon et al. 2007). Along the way, *Drosophila* researchers learned that Toll receptor can be activated not just by ligands encoded by the fly's own genome, but also by specific bits of an invading species, such as proteins in the flagellar tail of a bacterium or the double-stranded RNA typical of some viruses. Altogether, information gained via insect biochemistry, *Drosophila* genetics, and mammalian cell biology and immunology converged, and we gained a more complete picture of this important process than could have been achieved using any single approach alone.

The innate immune response, and its control by the Toll pathway, which became known as the "Toll-like receptor" or TLR pathway, turns out to be an evolutionarily ancient adaptation. Insects, mammals, birds, mollusks, jellyfish, corals, and even plants—all these multicellular living systems use versions of a TLR pathway as a means to detect and trigger responses to the invasive threats posed by bacteria, fungi, and viruses. They share the battle-readiness made possible by TLRs that induce expression of AMPs and other defense mechanisms that comprise the counter-attack. The findings revealed that the TLR pathway components evolved early in the natural

history of living organisms and have been preserved throughout long spans of evolutionary time; we share with other living creatures not only a common struggle against invasion by microbes but also the same cellular mechanisms for detection and response to the threat. Research into TLR pathways has also affected our ability to treat the immune system misfiring that results in human autoimmune disorders (Marshak-Rothstein 2006). The drug hydroxychloroquine, long used as an antimalarial treatment and used more recently to treat autoimmune disorders such as lupus and arthritis, turned out to be an inhibitor of human TLRs (Dunne, Marshall, and Mills 2011). TLR agonists, drugs that amp up TLR activity rather than quieting it down, also have clinical use. The human TLR agonist imiquimod is approved by the FDA for use as a treatment for genital warts (Hemmi et al. 2002), and at least one TLR agonist is used as an "adjuvant," a response booster, in vaccine formulations (Rappuoli et al. 2011). Additional TLR-targeting therapeutics are being tested in clinical trials or are in use for treatment of other immune system-related diseases, including cancer, chronic fatigue syndrome, and allergy (Bryant et al. 2015).

While some pursue TLR pathway research with understanding diseases and developing treatments foremost in mind, others continue to explore new territory. Some *Drosophila* researchers are examining the relationship between TLR pathway activity and space travel, which is known to put stress on the immune system. As part of these efforts, flies have been sent into space and subjected to altered gravity conditions here on Earth. Researchers then take a look at the impacts of these exposures on the flies' innate immune system, with the idea that gaining a better understanding of what happens to the immune system of flies in altered gravity conditions may help us make space travel safer for humans in the future (Taylor et al. 2014).

Unwelcome Guests

Many species of microbes that cause infectious diseases in humans, including bacteria, viruses, and fungi, have been studied using *Drosophila* (Panayidou, Ioannidou, and Apidianakis 2014). For human pathogens that elicit a response when introduced into flies (or cultured fly cells), the genetic approach can then be used to study "host factors"—genes in the fly host—that are used by the invader to gain access to host cells, make more of itself, and spread. *Drosophila* has also been used as a model or surro-

gate to study the infection by pathogens of other, harder-to-study insects, such as infection of mosquitoes by malaria-causing *Plasmodium falciparum* (Schneider and Shahabuddin 2000) or of sand flies by the leishmaniasis-causing trypanosome *Leishmania donovani* (Peltan et al. 2012). *Drosophila* has also been used as a model to study a trypanosome in the genus *Crithidia* that is a parasite of bees (Boulanger et al. 2001). What is learned about "host-pathogen interactions" in flies can lead to hypotheses regarding host-pathogen interactions in humans or in the insects normally exposed to these threats.

Notably, in the studies described above, researchers expose *Drosophila* to infectious pathogens they do not normally interact with in the wild. But we can remember and learn from the fact that *Drosophila* have a long evolutionary history of interaction with other organisms in the wild, including natural pathogens of the fly. Viruses provide just one example of the diversity of threats to which a fly might be exposed in the wild. In the 1960s and 1970s, a few types of fly-infecting viruses were identified, including *Drosophila melanogaster* sigma virus and *Drosophila* C virus (Jousset, Bergoin, and Revet 1977; Seecof 1968). More recent application of next generation sequencing to wild-caught flies, together with computational analyses specifically aimed at detection of viral or other non-fly sequences present in these samples, has led to the identification of many more types of viruses normally associated with some populations of wild flies (Webster et al. 2016; Webster et al. 2015). In addition to helping to identify the viruses themselves, these sequence-based approaches can also provide a survey of the proportion of wild flies infected by viruses. The results of one study suggest that about 30 percent of wild flies are infected with at least one type of virus, with a range of infection levels in a regional population of 10 percent to greater than 80 percent (Webster et al. 2015).

Studies of interactions between a host and a pathogen (or a predator, symbiont, or other interactor) that normally occur in the wild can reveal different information from studies aimed at using *Drosophila* as a model for infection by human pathogens (Keebaugh and Schlenke 2014; Kraaijeveld and Godfray 2009). Through studies of the natural pathogens of flies, we can learn what genes are relevant to the "co-evolution" of a host and its would-be guests. Some of these interactions transcend cellular level responses and cross into the realm of complex behaviors. An example is a behavior exhibited by flies in response to the threat of infection by parasitic wasps. When wasps are present in the fly's environment, female flies

will move from an alcohol-poor to an alcohol-rich food source and lay their eggs there. When the fly larvae hatch, they eat the boozy food, raising alcohol levels within that developing fly. As wasp larvae cannot thrive in an alcohol-rich environment, this behavior protects the female fly's offspring from the threat (Milan, Kacsoh, and Schlenke 2012; Kacsoh et al. 2013).

The Permanent Houseguest

Studying interactions that normally occur in the wild can also teach us about the impact of infection of a host with one organism on the chance that it becomes parasitized or infected by another (Thomas et al. 2000), as exemplified by the study of interactions among *Drosophila,* endosymbiotic bacteria, and parasitic wasps (Vavre, Mouton, and Pannebakker 2009) or among *Drosophila,* a bacterium called *Wolbachia,* and viruses. The information gained in such studies has, at least in one case, led to the development of a new strategy for limiting the spread of infectious disease. Some of these findings also challenge our ability to clearly define what is a pathogen and what is not. When my enemy is also the enemy of an enemy, is it a friend? *Wolbachia* bacteria provide an example of a case wherein the nature of the relationship between microbe and host is not straightforward. *Wolbachia* are unusual in that they that live inside the cells of a fly or other host species. They were first identified living in mosquito cells and were later detected in other insects, including many laboratory strains of *Drosophila. Wolbachia* exert surprising control over their hosts; they have the ability to override the host's normal genetic program, changing an insect's physiology, fertility, and behavior (Hilgenboecker et al. 2008). The fact that *Wolbachia* survive only within host cells raises a question. Given that an insect host will inevitably die, how do the bacteria spread and keep going? *Wolbachia* have evolved a clever work-around to the problem. They secure their futures by making sure that they are packaged inside developing eggs, so that they are passed along to the next generation. As a result of this transmission via eggs (but not sperm), *Wolbachia* have developed strategies that influence reproduction and bias conditions in favor of the survival of *Wolbachia*-infected and not uninfected fly offspring (Werren, Baldo, and Clark 2008).

In 2008, two groups reported evidence that infection of *Drosophila* with *Wolbachia* protects infected flies against would-be invading viruses (Teixeira,

Ferreira, and Ashburner 2008; Hedges et al. 2008). This is no doubt a self-interested behavior on the part of *Wolbachia*; keeping host cells healthy helps ensure survival of the bacteria as well. Nonetheless, this activity appears to provide a clear benefit to the fly, helping it stay free of viral infection, and might help explain why *Wolbachia* infection is widespread (Teixeira, Ferreira, and Ashburner 2008). Can this three-way relationship among an insect, a bacterium, and a virus end up benefiting humans as well? The answer might be yes. Researchers interested to reduce the spread of infectious disease by mosquitoes are trying to exploit this protective effect of *Wolbachia* to prevent infection of mosquitoes by human pathogenic viruses—a possible application noted by the two groups that first uncovered the interaction between the bacterium and viruses in *Drosophila* (Hedges et al. 2008; Teixeira, Ferreira, and Ashburner 2008).

Subsequent to the finding in *Drosophila,* others found that infection with a specific strain of *Wolbachia* of the disease vector mosquito *Aedes aegypti* appears to reduce transmission of the virus that causes dengue fever. The virus was detected in saliva of mosquitoes that were not infected with *Wolbachia,* but it was not in saliva of mosquitoes that were infected by the bacteria (Walker et al. 2011). Since mothers pass *Wolbachia* to their offspring, researchers expect that this protective effect might spread in a population, perhaps even in the wild, ideally reducing the proportion of wild *Aedes* mosquitoes that carry viruses or other infectious agents. By 2010, the method had already begun being tested in the field, with ongoing trials including the use of *Wolbachia* to help control dengue, Chikungunya, or Japanese encephalitis viruses, or *Plasmodium* (Walker et al. 2011; Moreira et al. 2009; Bian et al. 2010; Jeffries and Walker 2015). Introduction of *Wolbachia* into *Aedes* mosquitoes in Australia led to spread of the infection in the population (Turelli and Barton 2017; Hoffmann et al. 2011). The fact that *Wolbachia* are already found in many insect species, as well as the apparent natural limit of transmission of *Wolbachia* from mother to offspring but not beyond, are taken as suggestions that the approach might be relatively safe as well as effective.

Sensitivity and Resistance

Drosophila also provide a system in which to explore protection of a different kind. Given the negative impacts of some insects on human health and food security, there is great interest in learning more about how we

can effectively limit insect populations. One of the primary ways in which contemporary humans kill insects is through the use of chemical insecticides. Chemical insecticides fall into a variety of chemical classes and exert effects on different biological processes, including development, reproduction, and nervous system function. Despite their different modes of action, however, there is one thing all insecticides seem to have in common. Once a given insecticide has been used in a given region, chances are high that reapplication of a treatment that had been successful becomes ineffective upon subsequent trials. This phenomenon of "resistance" to insecticides has been appreciated at least since 1914, when entomologist A. L. Melander proposed that, rather than reflecting "mysterious adulteration" or chemical breakdown of the insecticide itself, perhaps the ineffectiveness of applying the same chemical to the same field a year later reflected the fact the insects themselves had changed (Melander 1914). More than a century later, insecticide resistance remains an issue that entomologists and others are trying to understand more fully and address. Notable, too, is the fact that resistance to insecticides is, as stated by L. Dävring in 1969, "only a special case of the far more general phenomenon of adaptability of populations to environmental changes" (Dävring 1969). Throughout evolution, insects have developed resistance to naturally occurring toxins such as metal ions, which are found at unusually high levels in some soil types, and to complex organic toxins made by plants or fungi. Thus, in learning about insecticide resistance, we can learn about natural resistance mechanisms as well.

In talking about insecticide resistance, it is tempting to say that insects "acquire" or "develop" resistance to an insecticide. But these terms apply only at the level of a population of insects, not at the level of individuals. An insect population can be said to have acquired or developed resistance over time upon multiple treatments with a toxin when the total population size bounces back to normal numbers following an initial decrease. What has happened at the level of individuals is very different. For an individual insect, "resistance" is not an attribute that can be gained but more a matter of chance. At the time an insecticide is applied, although most individuals are sensitive to the insecticide, a few lucky individuals might be naturally resistant, due to genetic differences that turn out to be relevant to the unanticipated new exposure. These few survive and procreate, whereas the others perish. As a result, the proportion of resistant versus sensitive individuals in the population shifts.

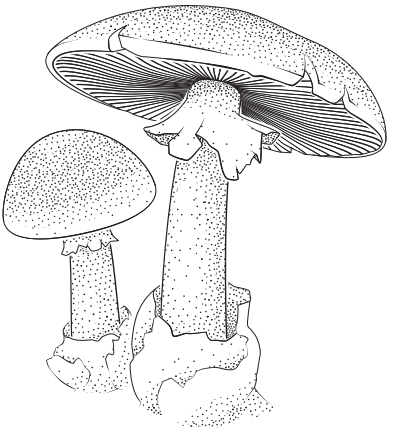
We can ask what specific molecular mechanisms make it possible for one insect to be resistant when others in the population are not. Working directly with the insects we intend to target with the insecticide would have obvious advantages in addressing this question. But doing so comes with significant challenges. Compared with *Drosophila,* no other insect is as well understood or so rich with resources for genetic analysis and other types of experimentation. As stated by T. Perry and colleagues in 2011, "As a precursor to rational pest insect control, the biology of the insect . . . must be well understood. It makes sense to initiate this research in the best model insect system, *Drosophila melanogaster,* and translate these findings and methodologies to other insects" (Perry, Batterham, and Daborn 2011).

An example of the utility of *Drosophila* is use in experimental studies aimed at identifying specific genetic changes associated with insecticide resistance, which in turn provides insights into the cellular mechanisms of resistance. Although it is now feasible to sequence sensitive and resistant strains of wild-caught insects to identify candidate resistance-associated mutations, it is not always obvious which of several candidate DNA differences is associated with resistance. One test that can provide definitive proof that a given mutation confers resistance is to introduce a putative resistance-associated mutant version of a gene into a sensitive insect, then ask whether the recipients of the altered version of the gene are now resistant. *Drosophila* can be used in such a test, even when the genes under study were identified in another species. Following identification of a mutation suspected of conferring resistance to the insecticide spinosad in the western flower thrip (*Frankliniella occidentalis*), researchers introduced a corresponding change into the sequence of the *Drosophila* gene *Dalpha6* and found that the change resulted in a 66-fold increase in resistance of *Drosophila* to spinosad, ending debate that had surrounded the capacity of a specific amino acid change to confer resistance to the chemical (Zimmer et al. 2016). For several other genes and insecticides, genetic studies in *Drosophila* helped identify what gene confers resistance. This is information that can be used strategically, as in co-treatment with insecticides for which the modes of resistance are known to be different, to improve the effectiveness of an insect control program.

Resistance at a Cost—or Not?

In addition to the development of resistance upon repeated application of a chemical insecticide, another apparent truism is that if a region is left untreated for a while, the proportion of resistant insects shifts back, with sensitive insects again outnumbering those that are resistant. Why does the population shift back? One hypothesis developed to explain this frequent observation is that mutations that lead to resistance also reduce the overall "fitness" of resistant insects as compared with wildtype—that is, under normal circumstances, the resistance mutations make animals less healthy or shorter lived, or reduce their rates of reproduction. In the 1950s, J. F. Crow, a pioneer in the field of population genetics, reported that *Drosophila* strains resistant to dichlorodiphenyltrichloroethane, better known as DDT, have a fitness disadvantage under normal conditions—providing direct evidence to support the hypothesis. Crow suggested that the fitness cost of DDT resistance accounts for the small proportion of resistant strains of insects in populations not yet exposed to the chemical (Crow 1954; 1957; Morton 1993). A fitness cost associated with resistance to a different insecticide, malathion, was later noted by R. S. Singh and R. A. Morton; in this case the resistance mutation is associated with developmental delay, wherein the resistant insects take longer to develop to adulthood compared with wildtype insects (Singh and Morton 1981). The idea that sensitive populations rebound because resistance comes at a cost is now a generally accepted concept that helps inform the design of insect control strategies.

As quickly as we think we have a handle on some concept, however, Nature delights the biologist by presenting an exception. *D. melanogaster* do not typically consume mushrooms of any sort. Thus, it seems especially unlikely that they would ever nibble on the highly toxic death cap mushroom, *Amanita phalloides*. It should follow, then, that if they do not eat death cap mushrooms, flies in the wild should not have developed resistance to alpha-amanitin, the deadly toxin made by these fungi. This appears to be largely true: most wild strains of *D. melanogaster* that have been tested are highly sensitive to the alpha-amanitin toxin. This is not true, however, for all strains. In the 1970s, D. E. Coulter, A. L. Greenleaf, and colleagues did a genetic screen to identify mutations in *Drosophila* that confer resistance to alpha-amanitin. They were able to find some mutant flies that were resistant to the toxin, demonstrating that *Drosophila* has the



The death cap mushroom, *Amanita phalloides*. Although *D. melanogaster* are not known to eat mushrooms, researchers have found wild strains that are resistant to the deadly toxin alpha-amanitin, which is made by *A. phalloides* mushrooms.

capacity to become resistant (Greenleaf et al. 1979). Still, the expectation was that no wild strains would have such a resistance. However, in 1982, J. P. Phillips, J. Willms, and A. Pitt reported the identification of three fly strains, collected in the wild from three different sites in Asia, that exhibit a naturally occurring resistance to alpha-amanitin. In a test of wildtype lab flies, a dose of 1.2 micrograms of alpha-amanitin in a standard fly food vial is enough to kill about half the flies in the vial. A much higher dose, as much as 35 micrograms per vial, was necessary to reach a level at which half the flies of these newly collected strains succumbed to the toxin (Phillips, Willms, and Pitt 1982).

One of the things we can do with *Drosophila* that is not as feasible with other model systems is to do nothing—for a long time. We can take a specific genotype of flies, or several, and, at a relatively modest cost keep them alive for years, allowing evolution to take its course in an isolated population. One such study was done with the three stocks of alpha-amanitin resistant flies originally collected from the wild. If there is a fitness cost to the genetic change conferring resistance to the fungal toxin, the expectation would be that, during the more than thirty-year period over which researchers cultured the flies on normal food (without the toxin), no flies or only a small proportion of flies in the population would still carry the resistance mutation. Instead, C. L. Mitchell, T. Werner, and colleagues found

in 2015 that all three strains had maintained the resistance allele, not lost it (Mitchell et al. 2015). The data suggest that, unlike mutations associated with DDT or malathion resistance, the mutation associated with resistance to alpha-amanitin does not confer a disadvantage. Moreover, the researchers found that when these fly strains were fed food with alpha-amanitin, fecundity went up (Mitchell et al. 2015). These data suggest that mutations conferring resistance to toxins do not always confer a disadvantage. Other researchers have similarly taken advantage of the ability to do long-term studies using flies, as in the so-called Dark Fly studies that placed fly cultures in the dark for years. One such study was initiated by Payne in 1907 (Payne 1910). Another, initiated by S. Mori in 1954, is still ongoing (Gelling 2016).

Resisting the Threat from Within

Not all threats are external. When the first version of a complete sequence of the *Drosophila* genome was released in 2000, researchers noted that a large proportion of the fly genome is made up of transposable elements, their remnants, or related sequences (Adams et al. 2000). The fly genome is not unique in being loaded with transposable elements; transposons are present in the genomes of many organisms, including our own. Because transposons can pick up and move around, they can be disruptive. Uncontrolled mobilization of transposons can have deleterious effects on chromosomal DNA, resulting in mutations, small or large deletions, inversions, and other changes that result in what is collectively termed "genome instability." As a result, mobilization of transposons can weaken fitness of an organism to the point of infertility or death, as well as create a situation in which new, deleterious mutations are passed along to a next generation at an alarming rate.

Perhaps not surprisingly, then, cells have mechanisms in place that keep these elements in check, blocking their ability to mobilize. Such factors are particularly important during formation of eggs or sperm, as scrambling the genome of these cells would be highly detrimental to the resulting offspring. Consistent with this, some of the genetic mutations that helped uncover the mechanisms by which cells suppress transposon activity resulted in flies with defects in fertility, for example, as associated with mutations in the *aubergine* or *Argonaute 3* (*Ago3*) genes. In the 2000s, we began to gain a mechanistic understanding of the complex molecular acrobatics that

keep these elements quiet (Senti and Brennecke 2010). Though the mechanisms turn out to be more universal, it was in large part research performed using *Drosophila*—including through the study of three genetic loci, *flamenco, piwi,* and *Stellate*—that led the way to understanding the mechanisms by which cells limit the potential of elements to mobilize (Han and Zamore 2014; Goriaux et al. 2014).

How do cells accomplish the task? Essentially, they use transposon sequences against themselves. Proteins capture RNA fragments made by transposons and pass them along, eventually cleaving them, in a process referred as the "ping-pong cycle" of transposon suppression (Goriaux et al. 2014; Senti and Brennecke 2010). More specifically, after an RNA molecule is transcribed from a transposon and exported from the nucleus into the cytoplasm, it becomes bound by Aubergine protein, together with a fragment of RNA that originates from the piRNA cluster. The transposon RNA then gets passed to the protein Ago3 and cleaved into a smaller fragment, and pairs up with RNA transcribed from the piRNA cluster. The piRNA cluster RNA then gets passed from Ago3 to Aubergine, where it is cleaved into a smaller fragment, ready to bind another transposon RNA, continuing the cycle of capture and destruction, and preventing transposon mobilization.

Once research in the fly made it clear what genes and activities to look for in other species, researchers were able to identify and characterize related proteins in human cells, including relatives of the Piwi protein that gives the piRNA system its "pi" name. The human versions of Piwi are referred to as the Hiwi proteins. Like Piwi in the fly, Hiwi proteins, along with proteins related to Aubergine and Ago3, help keep quiet the elements within our genomes that are otherwise ready and able to cause disruption. Even after the initial findings of similarity, studies in *Drosophila* have continued to inform and guide experiments in mammalian and other systems. Talking about Piwi and piRNAs at a research conference in 2015, P. D. Zamore stated that, after finding a particular activity of the piRNA system in flies, researchers went looking in mice and found that the same function was preserved in related proteins. He suggested that we would not have found this doing similar studies in mice because an additional function present in mice masked the activity. Based on the fly studies, however, researchers knew what to look for and once they did, the evidence showed that the same phenomenon occurs in mice.

Whether we speak of external or internal threats, be they natural or unnatural in origin, parallels between the mechanisms of defense and

resistance in *Drosophila* and other species abound. *Drosophila* research helps teach us how our cells, as well as those of our insect enemies, are wired up to respond to invasion, clean up would-be toxins, and suppress internal threats. Armed with this new information, we can not only learn fascinating truths about the biology of intracellular, organismal, and interspecies defenses, but also engage in noble pursuits—including development of new ways to treat autoimmune diseases, prevent the spread of infectious diseases by mosquitoes, and limit the destruction of food crops by insect pests.

CHAPTER EIGHT

BEHAVIOR

THE HUMAN BRAIN COULD BE DESCRIBED AS AN ORGAN THAT RECEIVES new information, integrates that input with information received in the past, and then elicits appropriate actions. But what an unsatisfying description for something that does so much, holds so many mysteries! Our brains help to control hunger and thirst, fear and love, sleep and wakefulness, the conscious or unconscious taking of a breath. They make it possible for us to form memories and associations, coordinate movement with intent, be alert in the face of danger. Our brains allow us to notice when our name has been spoken in a room full of competing noises or spot a friend far off in a crowd, and to distinguish stiff canvas from soft felt, the smell of a grapefruit from that of an orange, or the taste of Sichuan peppercorn from that of wasabi. It might defy reason to think that the complex brain activities and resulting behaviors, such as sensation, emotion, coordinated movement, sleep, and so on that seem to define us, define what it is to be human, could ever be reduced to the level of cell networks, individual "neurons" or nerve cells, or be even further refined, reduced to the level of specific biochemical activities performed by individual proteins encoded by genes. It might defy reason, too, to imagine that the *Drosophila* brain is capable of anything approaching a human brain's abilities. Yet through scientific investigation, we are finding that both are true. We can

reduce complex behaviors to the level of defined cellular and subcellular activities. And research in the fly—which exhibits an array of complex behaviors—is helping us to do so.

Currents and Channels

A relationship between the nervous system and electricity has long been noted; many of us observed the twitch of a dead frog's leg when hooked up to a battery in an introductory biology lab class, mirroring experiments reported by L. Galvani in 1791. Observing the animation of a frog's leg, we might ask, what is the relationship between the introduction of an electrical current and the activity of neuronal cells, which, in a living frog, control when its legs move, how often, and with what force? Are our brains like batteries, ready to emit currents that elicit twitches of activity that trigger responses such as movement, thought, sensory perception, and so on? The answer is largely yes. From early studies in which electricity was applied to dead or living tissue to more contemporary approaches such as the "patch clamp" technique (Neher, Sakmann, and Steinbach 1978), researchers have been able to detect the flux in current that is associated with the "firing" of a nerve cell, making these activities readable on a graph as spikes of responses along a timeline, and been able to manipulate these impulses, such as by application of drugs that cause nerves to fire continuously or cease firing at all.

Confirmation that nerves emit electrical signals raises new questions. After all, knowing an activity exists, even recording it, is distinct from understanding how that activity is generated. We do not have battery packs in our brains, nor do we plug ourselves in at night to recharge like a Smart Car after a commute. We can ask a number of questions about the cellular mechanisms underlying brain function. How are the neuronal "batteries" recharged or the flow of current controlled? How does this relate to the fine control of individual muscles, individual behaviors, discrete functions controlled by various brain functions, such that our activities occur in defined moments, controlled manners, rather than a frenzy of thought and twitch and activity? What genes and proteins are involved? Answers to these questions have come from many sources and result from the application of many different techniques. Some of these first answers to "What protein does that?"—answers that in turn help us answer the "How?" questions—were revealed through the study of *Drosophila*. Moreover, some of the

specialized techniques that have helped us answer these questions through direct study of the mammalian brain were initially developed in the fly.

Genetics Meets Neurophysiology

Channel proteins are proteins that span the plasma membrane, or an intracellular membrane, and create a passageway, like a pore on our skin, that can be opened and closed in a controlled manner. Channels are typically designed to let in only one or a few specific types of molecules. The batteries in our cars, flashlights, and computers require ions such as copper or lithium in order to generate electric charge. So, too, in our nerve cells, channels function to set up gradients of ions, creating voltage and allowing for the coordinated release of neurological signals by controlling the flow of ions from outside the cell in, or from one membrane-bound region to another within the cell. Our nerves rely on this fundamental activity, the establishment of ion gradients across membranes, in order to relay information within the extensive network of cells that comprise the central and peripheral nervous systems.

Prior to the 1980s, by applying biophysical and biochemical approaches in various systems, researchers identified channel proteins that act on sodium and calcium ions. Moreover, based on their measurements, researchers deduced that "voltage gated" potassium ion channels also existed. However, despite detecting the activity, researchers had not yet succeeded in identifying the specific protein sequence or corresponding gene that encodes this potassium ion (K^+) channel activity. Thus, how such a channel worked, in what cells they were expressed, and so on, were open questions.

Answers to the question of what specific proteins set up K^+ gradients across cell membranes came through the field of genetics. More specifically, they came through investigation of the *Shaker* mutant flies, originally identified in the 1940s and studied deeply by Y. N. Jan, L. Y. Jan, and others in the 1970s and 1980s. These researchers noticed that they could make flies behave similarly to *Shaker* mutant flies by applying a drug called 4-aminopyridine, which blocks K^+ channel activity (Jan and Jan 1997); a geneticist might say that treatment with the drug "phenocopied" the *Shaker* mutant effect. Based on this first hint, the researchers surmised that the *Shaker* gene encodes a K^+ channel. They eventually succeeded in isolating a DNA fragment containing the gene and demonstrating that the *Shaker* gene does indeed encode a protein with K^+ channel activity. Similar genes

were later identified in other organisms, again teaching us that once they arise, solutions to a problem tend to endure through evolution and get reapplied to similar problems. We now know that Shaker and other K^+ channel proteins are organized into clusters of four proteins, each of which has a lipophilic "bridge" that crosses the lipid-rich plasma membrane of the cell, a more "hydrophilic" end region that reaches out into the extracellular space (cell surface), and another hydrophilic end that extends into the cytoplasm of the cell. Viewed from outside the plasma membrane, a Shaker-type channel looks like a four-leaf clover, with a set of four leaflets—the voltage-sensing domains of each of the four component proteins—surrounding a central pore domain that comprises the channel through which the K^+ ions pass (Miller 2000).

Identification of this "first ever" K^+ channel "paved the way" to identification of additional channels in flies and in other species, including in humans (Frolov et al. 2012). Based on a shared phenotype, in 1969 W. D. Kaplan and W. E. Trout reported mutations in two additional genes, *hyperkinetic* and *ether-a-go-go* (Kaplan and Trout 1969), which in the 1990s were shown by B. Ganetzky and others to encode Shaker-related proteins (Warmke, Drysdale, and Ganetzky 1991; Chouinard et al. 1995). After availability of the fly and human genome sequences around 2000, it became obvious that within flies and humans (and other species) there are a large number of genes that encode K^+ channels. Moreover, more than 60 of the human genes that encode K^+ channels have been associated with disease. Through application of genetic tricks such as identification of mutant animals with similar phenotypes and identification of modifier mutations that genetically interact with the *Shaker* mutation, researchers identified additional K^+ channel-encoding genes in *Drosophila* and, significantly, built links between these genes and specific behaviors and processes. Shaker-type channels are now thought to be involved in various cell-level neuronal processes affecting behaviors as diverse as sleep, learning, flight, and mating (Frolov et al. 2012). They are also a common target of natural toxins. The venoms of scorpions and snakes, for example, as well as the toxins produced by sea anemones and cone snails, prevent the activity of K^+ channels by stoppering a channel "like a cork in a bottle," blocking the flow of ions and paralyzing the unfortunate victim (Wulff, Castle, and Pardo 2009).

In addition to diversity created by the existence of more than one *Shaker*-related gene within a species, additional diversity is created by the avail-

ability of different forms of RNA, through the process of "alternative splicing," such that a single K^+ channel gene can encode proteins that differ at one end or the other, or in the middle. *Drosophila Shaker* itself, for example, encodes 20 different RNA isoforms, suggesting that 20 different versions of the Shaker protein, differing from one another in specific regions, are encoded by this one gene. Assuming that similar variety exists for other *Shaker*-related genes in the fly, we can conclude that as a group these genes encode a very large number of unique proteins. The larger number of related genes in humans makes possible, at least in theory, an even larger set of variations on the theme. This leads to complexity at the cellular level. Different combinations of Shaker-related proteins and isoforms can be present in different cells, resulting in cells that respond to different stimuli or exhibit different responses to the same stimuli. Furthermore, channel diversity is not limited to Shaker-related proteins. Shaker-type K^+ channels represent one of nine subtypes, each of which can have multiple members: the K_v1, K_v2, K_v3, K_v4, K_v7, K_v10, K_v11, K_v12, and $K_{Ca}1.1$ subtypes. These are also referred to as the Shaker, Shab, Shaw, Shal, KCNQ, Eag, Erg, Elk, and Slo1-type K^+ channels, respectively (Frolov et al. 2012). With the exception of KCNQ (Wang et al. 1996), which was first identified in human cells, the first member of each of these other classes of K^+ channels was identified first in *Drosophila* (Frolov et al. 2012).

Further contributing to diversity among K^+ channels, within each subclass of channel protein, different members of the class can contribute to the four proteins that together make up a fully functional, cloverleaf-like channel (Jan and Jan 1997). We can view this complexity as reassuring. Most of us would like to think that our brains are complex and full of diversity, and indeed, at least when it comes to the makeup of K^+ channels, there is rich opportunity for variety, with differently composed channels exhibiting different activities. As with other genes and cellular activities, there is also opportunity for disruption, in the form of mutation, that leads to disease. Y. N. Jan, L. Y. Jan, and colleagues showed that mutations in the human EAG2 channel are associated with a subset of brain tumors, and have started testing the possibility that channel blockers can be effective in limiting tumor growth (Huang et al. 2015). Moreover, several human K^+ channels are associated with "channelopathies" such as those resulting in seizure disorders or ataxia (Wulff, Castle, and Pardo 2009).

"Transient Receptor Potential"

Just as the human heart can be monitored with an electrocardiogram or EKG, electrodes can be applied to a person's eye and responses to flashes of light, in the form of electrical signals, can be recorded—with steep cliffs of electrical response interrupting the flat line of the baseline signal. When such an electrical recording is done in the eye rather than in the heart, the recording is referred to as an electroretinogram, or ERG. Despite the small size of a fly eye, experts are able to hook up tiny electrodes to the eyes and record ERGs. In 1969, D. J. Cosens and A. Manning reported identification of a mutant fly strain, isolated spontaneously, for which the ERG is abnormal (Cosens and Manning 1969). In contrast to wildtype flies, which have a sustained response to a constant source of light, in these mutants the response is intermittent. The gene affected was named *transient receptor potential,* abbreviated *trp*. The *trp* gene was sequenced in 1989 (Montell and Rubin 1989). The protein encoded by the *trp* gene, TRP, is not another K^+ ion channel: the TRP family of proteins encode ion channels that shuttle other types of ions, such as calcium or sodium. But like K^+ channels, TRP can create an ion gradient, contributing to conductance of electrical signals in the eye, the brain, or other tissues.

As for the *Drosophila* K^+ channels, not one but several genes in the *Drosophila* genome encode TRP-related proteins. There are thirteen TRP-related proteins in flies, classified into seven distinct subgroups. Moreover, like the K^+ channels, TRP channels have been evolutionarily conserved. There are a total of twenty-seven TRP-related genes in the human genome, representing six of the seven subfamilies found in flies (Damann, Voets, and Nilius 2008). TRP family genes are "conserved in every metazoan organism that has been subjected to sequencing" (Montell 2011), and a TRP protein-encoding gene has been identified in the yeast *Saccharomyces cerevisiae* (Damann, Voets, and Nilius 2008).

But biologists are not satisfied with a mere catalogue of what exists. We see a list like this and must ask, so what do they do?

A Taste for More

One of the things TRP channels do is participate in the sensation of taste. Taste can be defined as an experience that combines detection of a chemical stimulus—sweet, salty, or bitter, for example—with an aspect of tempera-

ture, as is evident in our experience of a jalapeño pepper or a mint leaf. TRP channels turn out to be involved in both chemosensation and thermosensation. In flies and other species, our understanding of the roles of TRP channels in sensations such as taste opens the doors to detailed analysis of what constitutes taste at a molecular level and how we build associations with specific tastes. Given that we cannot ask a fly what it likes and does not like, how are studies of taste preference possible in a fly? One approach is to measure the amount of food a fly eats and ask whether flies eat more food of one flavor or another. This is, however, an imprecise assay, as food intake combines taste preference with behaviors controlled by other inputs, such as appetite. A hungry fly might eat more of a food it dislikes regardless of the taste; a less hungry fly might eat less even if it likes the food.

An alternative approach to testing taste preference takes advantage of the fact that the abdomen of a fly is translucent. Feed a fly food that has been dyed with a nontoxic food dye, let's say a blue dye, and the gut appears blue. Dye a different batch of food red, and the unnatural red within the gut can similarly be seen through the abdomen of an intact fly. And of course, dyes mix. Presented with blue- and red-dyed foods that are otherwise the same, a fly will feed on both with equal frequency, and the belly will turn purple. If the foods are different, however, and the fly has a preference for one over the other, causing it to eat more of, say, the red-dyed food as compared with the blue-dyed food, the gut will appear red. Importantly, the appetite of the fly does not affect the results; the ratio of blue- to red-dyed food is measured, not the total amount.

A study by Y. V. Zhang, C. Montell, and others took advantage of this test to explore a behavior that couples taste with another stimulus. Specifically, the group was interested to determine whether flies could get over an aversion to a taste, in this case camphor, given some incentive, such as dosing the camphor-containing food with five times the amount of sugar provided in the alternative food mix (Zhang et al. 2013). What did they find? For one thing, the flies could unlearn their dislike of camphor, which is detected in flies by a TRP family protein called TRPL. After being fed for a while on a sweet, sugary, but camphor-containing food source, levels of TRPL protein went down, desensitizing the flies to the bad taste, so that when they were then tested for taste preference, they did not shy away from the camphor-containing food, whereas flies fed a normal diet did. At the level of electrical signals, the blips of activity that would normally result

when a fly was presented with camphor-containing food also went away; the flies were desensitized at a molecular level. The research group then asked whether the effect was enduring and found that it did not last long. If the desensitized flies were fed normal food for a while, levels of TRPL went back up, the electrical blips were again detected when the flies were newly exposed to camphor, and the flies would turn their proverbial noses up at camphor-containing food, just as they had before the testing began. An important aspect of this work is that, although flies appear to dislike camphor, it is not, at the levels delivered in the study, toxic to the flies. For substances that are toxic, such as the bitter compound quinine, desensitization was not observed—to grossly anthropomorphize, we can say that flies are smart enough not to let themselves get used to the taste of a poison.

TRPs as Receptors for Sensation

Taste combines thermosensation with chemosensation. Like taste, touch also includes a component of thermosensation. The sensation of shaking hands with someone who has just stepped in the door on a cold winter day is very different from the sensation of being touched by the same fingers after they have warmed. Rather than combining thermosensation with chemosensation as in taste, however, touch combines thermosensation with mechanosensation. A firm handshake is different from a weak one, and the feeling against the skin of something hard like a paperclip is distinct from the feel of a fluff of cotton. TRPs turn out to be involved in sensing all three of these stimuli, thermosensation, chemosensation, and mechanosensation (researchers think mechanosensation might be the most ancient of these functions, as the sole TRP channel in yeast detects mechanical force [Zhou et al. 2003]). We can add "hygrosensation" to the list of stimuli sensed by TRP channels as well. Flies with a mutation in the TRP protein-encoding gene *water witch,* for example, revealed the normal ability of a TRP channel to detect humidity (Liu et al. 2007). Individually, the TRPs expressed in sensory cells of the fly serve as readouts of specific sensations; collectively, they allow a fly to experience perturbations or experiences to which we might assign a single integrative name—savory or pungent, fluffy or sharp, comfortable or swampy.

Notable, too, is the fact that TRP proteins do not function only in the brain. As sensors of touch, TRPs are found in many tissues of the body. TRP channels present on the bristles of a *Drosophila* wing can detect mites.

Indeed, the sensation of a mite crawling on the wing triggers a circling flick of the fly's leg that knocks the mite off—a response that will occur even in a decapitated fly, telling us something about which nerve cells and connections are and are not required for the response (Li, Zhang, et al. 2016). TRP channels have roles in a variety of tissues in humans as well. Disease-related studies of TRPs include studies of how TRP channels are related to chronic cough, which might constitute an over-response of TRPs in the lungs to a negligible or nonexistent touch signal. Results that support this idea include the finding that human TRPs are at increased levels in the relevant tissues of patients with chronic cough. For this reason, a few antagonists of TRP channels are being explored as potential drug treatments for chronic cough (Bonvini et al. 2015). In addition, changes in the DNA sequence or expression of TRP genes are associated with heart disease, including developmental defects, arrhythmia, and cardiomyopathy (Yue et al. 2015), and with diseases affecting the kidneys, bladder, skin, or other organs (Nilius, Voets, and Peters 2005).

Flies and the Study of Complex Behaviors

Already in 1907, researchers reported studying the behavior of fruit flies in response to odors (Barrows 1907). In the late 1960s and 1970s, the study of behavior entered a new era in which forward genetic screens in *Drosophila* provided biologists with a sense of what behaviors can be altered via mutation of single genes, and insights into the processes those single-gene mutations disrupted. Through the efforts of S. Benzer and others, we learned that the spectrum of behavioral defects that can be observed in mutant fly strains is surprisingly rich. Researchers identified mutant flies with an inability to remember that a given odor is associated with delivery of an electric shock, something normal flies can do; flies that fail to move opposite the pull of gravity or toward a source of light as they would normally do; flies that fail to take flight in response to some threatening stimulus even though the wings are perfectly capable of doing so; male flies that are unsuccessful in their courtship of females due to improper execution of the usual male mating behaviors; flies that have seizures following exposure to an anesthetic or following a mechanical disruption like tapping the culture vial on the counter (known as the "bang sensitive" phenotype); and flies that become paralyzed upon exposure to a temperature tolerated by normal flies (Benzer 1971).

These disruptions in whole programs of complex behaviors, each associated with disruption of a single gene, challenged the long-held idea that complex behaviors are controlled by mechanisms too complex to reduce to single genes or to tease apart from environmental inputs. Reports of behavioral mutant flies "prompt provocative thinking" (Drayna 2006) about the contribution of genes to complex behavior-related concepts such as sexual orientation, addiction, consciousness, and personality. As in the case of taste preference, the idea of exploring these topics in flies also raises questions regarding how the research can be performed. How do we define and study normal behaviors? What would be the phenotypic assay if a researcher undertook a genetic screen to identify mutations that disrupt these behaviors? Can these assays be designed in such a way that they generate quantitative, rather than qualitative results?

Careful observation of free-roaming *Drosophila* suggests that flies spend about half their time performing repetitive behaviors such as grooming themselves with their legs, running, or standing idle (Berman et al. 2014). When challenged with stimuli such as food odors, potential mates, or the looming presence of a predator, flies exhibit a broader range of behaviors. Many examples of normal behaviors of the fly can be analyzed using the type of quantitative assays that facilitate meaningful research, including the following. *Activity*: To learn how active a given genotype of fly is, place flies of that genotype on a grid and count how many times they cross any line on the grid within a fixed period of time (Neckameyer and Bhatt 2016). *Aggression*: Place a pair of male flies in an "arena," a contained area in which they are in competition for food or a mate, and observe the type and number of aggressive advances the males make toward one another (Anholt and Mackay 2012; Edwards et al. 2006; Yurkovic et al. 2006). *Attraction or aversion to odors*: Count the proportion of flies, or time spent by one fly, far from or near to a source of odor, such as on a cotton swab placed in a vial (Neckameyer and Bhatt 2016). *Avoidance of predators*: Monitor "wall following," the tendency of a fly to spend more time close to the wall of a chamber than in the middle, a behavior that presumably makes it less susceptible to predation (Mohammad et al. 2016). *Courtship*: Look for "chaining," observed when especially randy flies form a kind of conga line in which each fly is attempting to court the one in front of it (Zhou et al. 2015; Simon and Dickinson 2010); or observe stereotypical components of the male courtship ritual, such as singing, tapping, or curling

(Neckameyer and Bhatt 2016). *Detection of motion and "orienteering"*: Tether a fly from above and give it a small sphere to walk on, then present the fly with visual patterns, such as parallel or perpendicular lines, and ask in what direction it walks (Silies, Gohl, and Clandinin 2014). *Escape or evasion from predators*: Present a looming predator, or cast a moving shadow to mimic one, and ask if flies stop moving (Angus 1974) or attempt to jump away (Peek and Card 2016). *Intoxication with alcohol*: Expose flies to ethanol vapor and collect them as they stagger and fall from loss of coordination (Leibovitch et al. 1995). *Learning and memory, negative associations*: Ask if the fly moves away from an odor previously paired with a negative stimulus such as a pathogen (Das, Lin, and Waddell 2016). *Learning and memory*, *positive associations*: Ask if the fly moves toward an odor previously paired with a positive stimulus such as food or water (Das, Lin, and Waddell 2016). *Learning and memory, teaching others*: Expose flies to parasitic wasps, then monitor responses, which reportedly can be remembered and "taught" to flies that never encountered the predator (Kacsoh et al. 2015). *Past experience as an influence on current behavior*: Compare the subsequent behaviors of "winner" versus "loser" males after they are induced to fight (Yurkovic et al. 2006). *Preference for light or darkness*: Place a fly in a box that is half clear (letting in light) and half opaque (dark), and count the number of times the fly moves from one area to the other and the proportion of time spent in the light (Neckameyer and Bhatt 2016).

The "Fabulous Test-Bed" of Neuroscience

The tests that allow *Drosophila* researchers to study various aspects of behavior build upon one another, allowing us to explore increasingly complex questions. As noted, once we have an assay for taste, we can go on to develop tests that build upon those assays—exploring memory, for example, by asking whether the fly makes a lasting association between a good or bad taste and some other stimulus. As described, we can take the assay further, asking to what extent a fly will ignore a negative stimulus if it is associated with a reward, and for how long the fly remembers to associate two experiences. Similarly, once we have a boxing-match style arena in place to test aggression, we go on to ask questions of the winners and losers. For example, we can ask whether a winner or loser is more or less

likely to win a next battle, self-medicate with food or alcohol, or change its behavior toward other male flies or towards female flies. Foundational genetic studies by S. Benzer in the area of circadian rhythms (Konopka and Benzer 1971)—another "first in fly" research area—led to a clear understanding of how circadian rhythms are controlled at a molecular level, as recognized in 2017 by the awarding of the Nobel Prize to J. C. Hall, M. Rosbash, and M. W. Young (Nobel Media 2017). Researchers are expanding this new understanding by exploring the interaction of sleep (or lack thereof) with other behaviors. Altogether, over the past several decades, *Drosophila* neuroscientists have shared information, built upon one another's ideas, and developed new assays, moving us toward an increasingly detailed and accurate understanding of the molecular mechanisms that underlie animal behavior.

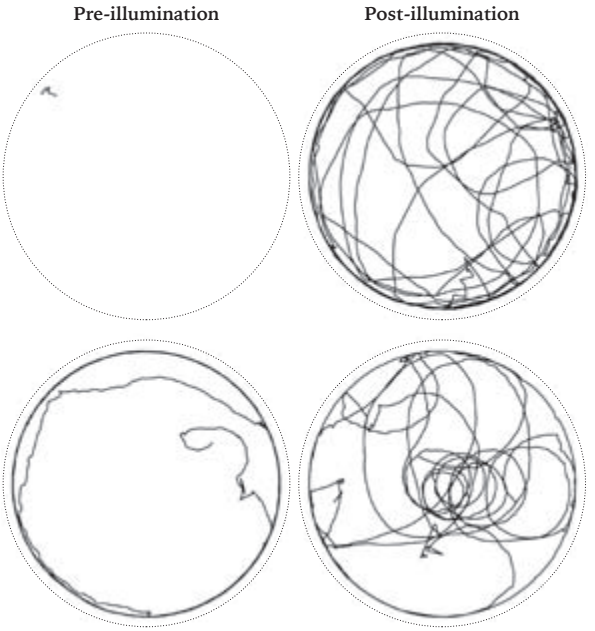
The *Drosophila* brain has been the subject of intensive and large-scale studies of other kinds as well, spurred on by the development of other types of tools and technologies. The tiny fly brain can be dissected, mounted, sliced, and subjected to fluorescence and electron microscopy, providing detailed views of brain regions, individual cells, and the location of specific proteins within them. In efforts that parallel the sequencing of genomes in both scale and ambition, researchers in the United States and Taiwan have mapped the positions of the more than 100,000 neurons in the fly's brain, examining the location of nerve cell bodies and their branch-like connections to one another (Aso et al. 2014; Wolff, Iyer, and Rubin 2015; Chiang et al. 2011; Shih et al. 2015), efforts that build upon earlier studies (Hanesch, Fischbach, and Heisenberg 1989). Even with contemporary image analysis technologies to help things along, this detailed mapping of the fly brain has taken the efforts of many, working for years, to accomplish. It is an unprecedented achievement in anatomical description, a feat that ought to garner the attention paid to the early polar expeditions or the Apollo space missions. Like those projects, the endeavor to map the fly's brain in its entirety and in detail took resources, teamwork, an adventurous spirit, and a forward-thinking mindset. Mapping model organisms' genomes helped to accelerate the human genome sequence project by providing simpler systems with which researchers could test approaches to sequencing and sequence data assembly; mapping the fly brain in its entirety, particularly when combined with genetic study and cell biological manipulation that paint function onto form, can help guide similar efforts to map and

understand the human brain. As with any other expedition into unknown realms, there is value in knowing what to look for—what cell types might be present in what subregions, what cues and patterns of expression might be present, what connections might link one region to another, as well as value in knowing what tools are required to accomplish the goal.

Some of the technologies applied to flies cross into the realm of science fiction. In the 1970s, C. A. Poodry and colleagues reported a temperature-sensitive mutation in a gene called *shibire,* which encodes a protein called dynamin (Poodry and Edgar 1979; Poodry, Hall, and Suzuki 1973). Dynamin is required for cellular events that are critical to neuronal activity. In 2001, T. Kitamoto showed that a temperature-sensitive mutant version of *shibire* can be used as a tool by expressing the mutant form using the Gal4-UAS approach. The effect of expression, when combined with exposure to increased temperature, is to inactivate whatever specific neurons of the fly brain express Gal4 and thus the mutant form of *shibire* (Kitamoto 2001). The approach allows for "rapid and reversible perturbation of synaptic neurotransmission" (Kasuya, Ishimoto, and Kitamoto 2009). In other words, researchers can stop specific neurons from firing, and then observe the outcome—an approach with parallels to the genetic approach.

In 2005, S. Q. Lima and G. Miesenböck reported a technology that does the opposite, allowing us to turn neurons on in an intact, living animal (Lima and Miesenböck 2005). This technology, which was applied first in the fly and became known as "optogenetics," built upon results of an earlier study in which a light-sensitive fly protein was expressed in mammalian cells (Zemelman et al. 2002). Both approaches—the turning off of genes using a *shibire* mutant gene or the turning on of neurons using optogenetic technology—can be combined with behavioral assays. Turning off or on of neurons in one region or of one specific neuronal cell subtype might affect learning and memory; applying the same perturbation in another region might affect a very different neurological process or behavior, such as taste, vision, or locomotion.

In presenting the optogenetic approach in 2005, Lima and Miesenböck reported the ability to stimulate different sets of neurons and observe behaviors this stimulation elicits—from jumping and flight to speed walking, with some inactive flies responding by becoming active and active flies slowing down. In 2014, D. E. Bath, B. J. Dickson, A. D. Straw, and colleagues reported a related approach they named FlyMAD, for Fly Mind-Altering



Movement trajectories of flies before and after genetically targeted photostimulation of dopaminergic neurons. The paths of two different flies in a cylindrical glass arena before (left) or after (right) activation of specially engineered neurons with light. Reprinted from S. Q. Lima and G. Miesenböck, 2005, "Remote control of behavior through genetically targeted photostimulation of neurons," *Cell* 121 (1): 141–152, Figure 6, A–D, with permission from Elsevier. Copyright © 2005 Elsevier Inc. All rights reserved.

Device, in which an automated video system can detect an individual fly and deliver a warming laser pulse that similarly can activate a discrete set of neurons (Bath et al. 2014). Fly biologists like to tinker with existing technologies, making them easier and more powerful. Further refinements and variations on the theme of neuronal control and monitoring have been reported and are likely to continue to appear. Moreover, some technologies are tried out first and refined in the fly, and then introduced into use in other systems. Since it was first demonstrated in the fly, optogenetic technology has been applied extensively not just in flies but also in fish, primates, and other organisms as part of diverse neurological studies. Altogether, the fly has been described as a "fabulous test-bed for new genetically encoded tools," a kind of playground or beta test site for the development of leading-edge technological innovations that let us ask new questions and determine

answers with better efficiency and increased precision (Owald, Lin, and Waddell 2015). Some of these, such as innovations in microscopy, improve our ability to make precise observations; others, like optogenetics, allow us to experiment. Together, they inform.

Drunk Flies

Can't walk straight, hits on anything that moves, parties hard, crashes harder. As much as these sound like behaviors that might be exhibited by a bunch of twenty-somethings at a nightclub, the same signs are characteristic of changes exhibited by fruit flies exposed to alcohol in a lab. The commonality between fly responses to alcohol and our own responses, at the cellular and behavioral levels, is another aspect of normal fly behavior being exploited by Drosophilists. Like us, *Drosophila* willingly consume alcohol and can use it as an energy source, breaking it down metabolically into sugars. Like us, wild *Drosophila* have a long natural history of associating with alcohol. Their native diet is rotting fruit, after all, and much of that rot is happening via fermentation, with alcohol the natural byproduct. Moreover, also like us, *Drosophila* can get inebriated. When exposed to alcohol, flies get hyperactive at first, and then, as they consume more alcohol, get sluggish, stumble around, and eventually topple over (Guarnieri and Heberlein 2003). Their courtship behaviors also change, with inhibitions removed (Lee et al. 2008). To measure differences in the sensitivity to alcohol of different genotypes of flies, researchers designed a multitiered device called an "inebriometer," in which drunk flies fall to the bottom of the device whereas sober flies can keep themselves upright on higher tiers (Cohan and Hoffmann 1986; Weber 1988; Moore et al. 1998). Refinement of the design of the inebriometer led to improvements that make the device appropriate for testing anesthetics as well as alcohol vapor, extending its utility (Dawson et al. 2013).

Although the idea of an alcohol-addicted fly might be hard to grasp, U. Heberlein and others have demonstrated that under the right initiating conditions, flies show several signs consistent with the criteria required of mammalian models of alcohol dependence, and the same molecular pathways appear to be relevant to alcohol in both flies and mammals (Devineni and Heberlein 2010). The rich array of established behavioral assays, from courtship to aggression to taste preference, makes it possible to ask what changes occur in individual and social behaviors of flies following

exposure to alcohol, including what a fly will put up with in order to get its next drink. Behavioral assays can be layered with genetic and biochemical studies, as well as with manipulation of specific brain regions or neurons, to provide a picture of the near-term and long-term effects of alcohol on the fly brain (Devineni and Heberlein 2010).

What we learn in the fly can likewise be combined with information gained from human genomic analysis of susceptibility to alcohol dependence. The results of one study correlated alcohol dependence with the human gene *XRCC5*. Perturbation of the function of the equivalent gene in the fly, *Ku80,* led to an alcohol-resistant phenotype in flies, supporting the idea that the gene's normal function is relevant to alcohol or alcohol-related behaviors (Juraeva et al. 2015). In an earlier study, an alcohol sensitivity phenotype was observed in the context of reduced function of a fly ortholog of another gene associated with alcohol dependence, the human *AUTS2* gene, an observation that also supports the idea of an association between mutation of *AUTS2* and alcohol dependence (Schumann et al. 2011). The opposing alcohol-related phenotypes observed in flies—resistance in one case, sensitivity in the other—might turn out to reveal something about the two candidate human genes. In another study, researchers did a genetic screen for alcohol-related fly phenotypes, identified the genes associated with those phenotypes, and then looked at what was known about related genes in humans; the study led to identification of an increased risk of alcohol addiction in people with specific variant forms of the human *RSU1* gene (Ojelade et al. 2015). The availability of *Drosophila* as a system in which alcohol-related assays can be performed with wildtype and genetically perturbed strains helped these research groups focus in on the most relevant human gene candidates and develop hypotheses as to how the genes are relevant.

Are Flies Conscious?

Some studies in *Drosophila* push the limits of what we might think a small organism like a fly is capable of when it comes to behavior, as well as what types of behaviors or related brain activities can be reduced to the level of individual genes, molecules, and mechanisms. Studies aimed at exploring consciousness provide an example. When we sleep, or when we enter the "drug-induced, reversible state of decreased responsiveness" brought on by

general anesthesia (Zalucki and van Swinderen 2016), we move from a conscious, awake state to an unconscious state. Does it follow, then, that since a fly has a sleep cycle and can be put under with anesthesia, we can consider the awake and undrugged fly to be conscious? In 1928, one entomologist speculated that "the insect may be a conscious being," but if so, "its consciousness be dim and feeble as compared with that of a vertebrate" (Carpenter 1928). Much more recently, B. van Swinderen has stated that "if consciousness is self-awareness, then flies are very unlikely to be conscious in the sense that we humans appreciate the concept" (van Swinderen 2005). Van Swinderen is quick to note, however, that this does not mean the fly cannot be useful in studies related to consciousness. He suggests that we should "be agnostic about an insect's potential subjective experience and instead tackle quantitative variables associated with changes in consciousness," such as by using flies in mechanistic studies of consciousness-related phenomena. These could include the study of sleep and anesthesia, as well as the phenomenon of "selective attention," which manifests in flies as persistent watching of one object even when other objects are in view, such as when a fly that has recently defeated another fly in a bout of aggression keeps watching the loser even though other flies are around (van Swinderen 2005).

Work in flies and other model organisms provides insights into the different molecular targets or cell responses associated with anesthesia as compared with normal sleep (Zalucki and van Swinderen 2016). Studying three systems in particular—the worm, which has 302 neurons; the fly, which has some number of neurons in the range of 100,000; and we humans, which are estimated to have billions of neurons—provides a graded series from simple to complex. These studies allow researchers to learn commonalities and differences regarding the normal processes, mechanisms, and activities of a brain. *Drosophila* are also being used as a model for understanding the fundamentals of personality, which can be defined as the making of predictable, individual choices that are not heritable (not the result of a genetic difference) (Kain, Stokes, and de Bivort 2012), and the fundamentals of an emotional state, wherein an experience encountered in one moment influences the next behaviors (Anderson and Adolphs 2014).

Consciousness, personality, and emotional states—in flies? What an intriguing time we are in. We have tools for turning genes and individual neurons off or on; detailed maps of the fly brain; sophisticated assays; and

a growing sense of the molecular mechanisms that underlie complex behaviors. We can only begin to imagine what new information might be layered upon what is already known as research on the normal behaviors of flies continues. We can predict, however, that this information will help us understand how our brains integrate inputs and elicit complex behaviors.

CHAPTER NINE

COORDINATION

TAKE A CLEAR GLASS VIAL OF NORMAL ADULT FRUIT FLIES, TAP IT GENTLY on the table, and the flies will exhibit a stereotyped response: they will remain at the bottom, stunned, for a few milliseconds, then right themselves and quickly begin walking upward along the sides of the vial, toward the stoppered top. The formal name of this test is the "negative geotaxis assay," but in the day-to-day course of research, fly biologists refer to it as the "climbing assay." This test is often done by hand, but attempts have been made to automate it, using a mechanical device to produce the gentle tap of the vial, a video capture setup to record the flies' responses, and custom video analysis software to analyze the images and report on the success or failure of individual flies to reach a given level on the vial within a fixed number of seconds (Madabattula et al. 2015; Podratz et al. 2013). In any given trial, not every fly will reach the top of the vial at exactly the same time. Moreover, not all the flies will exhibit the climbing response every time they are tested. However, given 30 seconds or so to respond, most young, fit, wildtype flies will reliably react the same way, quickly walking upward after the disruption.

Just as we slow down and get a little clumsier in old age, so, too, wildtype flies start to perform poorly on the climbing assay as they get older. For certain mutations, the effects of aging on performance in the assay are

more pronounced, such as a failure of older flies to climb even halfway up the vial, poor performance beginning at a younger age, or both. As with any good scientific assay, the climbing assay can be "controlled"—with a set of age-matched, wildtype, and untreated flies tested alongside experimental genotypes or drug-treated flies, providing an essential comparison point. One of the things that is both utilitarian and satisfying about the climbing assay is that it allows a complex behavior involving coordination of several systems—the brain, the peripheral nerves, and the muscles—to be reduced to a single numerical value. Results of the climbing assay are often presented as the percentage of flies that reached some height within some fixed number of seconds after the tap. The percentage obtained for control flies can be compared with results obtained for experimental flies, and the numbers analyzed statistically and plotted on a graph, providing a quantitative readout of a complex biological phenomenon.

This assay exemplifies one of the most powerful applications of *Drosophila* for biomedical research: the study of neurological function in an intact living system and over the full course of its lifetime. Combined with studies at the leading edge of technology—from sophisticated microscopy techniques to multifactorial molecular genetic manipulations—assays like the climbing assay are helping to uncover first insights into neurological function, and, perhaps even more importantly, dysfunction.

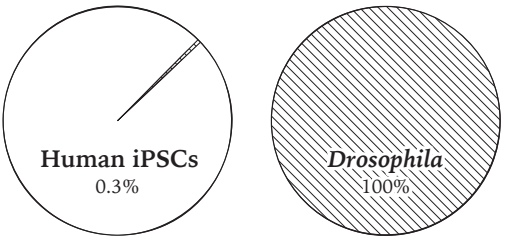
The Shaking Palsy

In 1817, J. Parkinson published a report of what he called "the shaking palsy," the disease we now know as Parkinson's disease. He described the "unhappy sufferer" of the disease as experiencing "involuntary tremulous motion," with an inevitable progression to a state in which "the trunk is almost permanently bowed, the muscular power is more decidedly diminished, and the tremulous agitation becomes violent" (republished as [Parkinson 2002]). Parkinson's disease is one of several "neurodegenerative diseases" in which, as the name implies, nerve cells degenerate and die, leading to the loss of specific activities. Other neurodegenerative diseases include Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, prion disease, and a set of diseases known as "tauopathies" due to the shared relevance of the human TAU protein to disease pathology.

One of the desires Parkinson expressed in his 1817 article was to excite others "to extend their researches to this malady" (Parkinson 2002). Many

have answered the call but progress is modest. There are several therapeutic strategies available to help alleviate symptoms in patients diagnosed with Parkinson's disease (Oertel and Schulz 2016; Nutt and Wooten 2005). Despite the span of two centuries since Parkinson's report, however, Parkinson's disease remains difficult to diagnose, particularly in early stages, and incurable, with available treatments typically providing at best palliative and temporary relief of symptoms rather than reversing or preventing the deadly decline (Oertel and Schulz 2016). Sadly, this is also the case for Alzheimer's disease, Huntington's disease, ALS, and other neurodegenerative diseases. Given the high personal and societal burden of these diseases, researchers are more eager than ever for new information—answers to the question of how these diseases begin and progress—that can help in the development of new drugs or treatment strategies. Even help with early diagnosis would be welcome. Parkinson's disease is typically diagnosed based on extrinsic symptoms such as a hand tremor or a particular change in the way a person walks (Nutt and Wooten 2005). Physicians would welcome methods that improve upon this, such as identification of Parkinson's disease "biomarkers" or indicator proteins in the blood, urine, or saliva. Identifying signs of disease earlier, and making a definitive diagnosis, are both things that can help patients and their families cope with disease and develop a treatment strategy.

Though it might seem surprising, *Drosophila* is among the research systems contributing new information relevant to Parkinson's disease and other neurodegenerative diseases. Many of the genes implicated in Parkinson's disease have orthologs in flies, opening the door to application of the genetic approach. Various engineered strains of *Drosophila* serve as "disease models," with specific genotypes of flies exhibiting "symptoms" or phenotypes that recapitulate features of the disease at the level of cellular, physiological, and whole-animal outcomes. Indeed, the climbing assay is one of the tools that is being used by *Drosophila* researchers to study Parkinson's disease. *Drosophila* models of Parkinson's disease offer a number of advantages as compared with other approaches to the study of human diseases, such as the use of human iPSCs. Chief among these virtues, perhaps, is that the study period available for *Drosophila* models of diseases like Parkinson's disease represents the entire lifespan of the organism—a highly relevant attribute given the relationship between the onset and severity of Parkinson's disease symptoms and aging—whereas human iPSCs, though having the advantage of being human in origin, can typically be cultured in the laboratory for only a few months, representing a small



Study period as a percentage of total lifespan of the organism. Human induced pluripotent stem cells (iPSCs) can be cultured for only about three months, a small fraction of the total human lifespan. By contrast, *Drosophila* can be monitored across the entire adult period. Doing parallel and complementary studies in both iPSCs and *Drosophila* can help researchers capitalize on the advantages of each.

fraction of our lifetime. An effective approach is to use different experimental systems in parallel or in series, capitalizing on the strengths of each, with information gained from studies in one organism or cell type helping to inform and guide further studies in the other.

A Cellular Garbage Strike

In 1998, T. Kitada, N. Shimizu, and colleagues reported the cloning of a human gene that they called "parkin" (and was given the official human gene name *PARK2*) that is associated with a rare inherited form of Parkinson's disease (Kitada et al. 1998). There is a stretch of about 70 amino acids at the beginning of the PARK2 protein that has similarity to short proteins called Ubiquitin proteins. There is also a region with similarity to another type of protein, RING-finger proteins. A molecular process involving Ubiquitin had previously been identified in humans and other organisms, including plants. The process is a tag-and-grab system, wherein damaged proteins or other biomolecules are recognized; one or more copies of the small Ubiquitin protein are "ligated" or attached to these damaged proteins; and this Ubiquitin-tagged "cargo" is then shuttled into the proteasome, a large multi-protein complex that functions as a garbage disposal, breaking proteins up into smaller, recyclable parts (amino acids).

The similarity between PARK2 and Ubiquitin suggested that PARK2 might similarly be involved in the Ubiquitin-mediated recycling system (Kitada et al. 1998; Nussbaum 1998). The fact that specific cellular structures called Lewy bodies, which are associated with Parkinson's disease but

not with normal cells, appear to contain Ubiquitin and proteasome components further supported the connection between this system and Parkinson's disease. However, the observation that the PARK2 sequence diverged in some ways from the protein to which it was being compared, including the presence of an additional region with a different predicted biochemical function, led researchers to speculate that PARK2 and Ubiquitin might have related but not identical roles. Altogether, the identification of *PARK2* provided hints but no firm answers in the hunt for components of "what will certainly be a complex pathogenetic pathway" related to Parkinson's disease (Nussbaum 1998). The story was similar for other Parkinson's disease genes, as well as genes related to Alzheimer's disease. Hints regarding function could be found, but a fuller picture of what molecular and cellular mechanisms were relevant was missing. Adding to the difficulty of studying some neurodegenerative diseases and their genetic links, attempts to recapitulate disease symptoms via disruption of the *Parkin* gene in mice did not result in a clear-cut disease model; the "mouse models fail to replicate many key features of Parkinson's disease" (Dawson, Ko, and Dawson 2010).

Some researchers turned to *Drosophila* for answers. In 2000, M. Feany and W. Bender reported that introduction of the human gene encoding alpha-synuclein (*SNCA*) into *Drosophila* resulted in a fly model of Parkinson's disease that "recapitulates the essential features of the human disorder," including age-related loss of neurons and locomotor dysfunction (Feany and Bender 2000). Versions of the human gene associated with a dominant inherited form of Parkinson's disease resulted in more severe Parkinson's disease-related effects than did introduction of the wildtype human gene, for which there is no apparent related gene in the *Drosophila* genome. The paper was "warmly welcomed" in the field (Rincon-Limas, Jensen, and Fernandez-Funez 2012), with medical experts such as M. F. Beal recognizing that the fly model would "allow the rapid characterization of enhancer and suppressor mutations—an important advantage" (Beal 2001). Other reports followed, including from N. Bonini and colleagues, who observed neurotoxicity induced by alpha-synuclein, again consistent with what is observed for Parkinson's disease in humans (Auluck et al. 2002). Unlike the case for *SNCA*, there is a gene in *Drosophila* that encodes a protein related to *PARK2*, making genetic tests in the fly even more straightforward to perform and interpret. The fly ortholog of *PARK2* was named *parkin,* after the name given to the human gene by Kitada and colleagues.

Othologs of several other Parkinson's disease-related genes are found in the fly genome, including orthologs of human *PARK5* (official human gene name *UCHL1*), *PARK6* (*PINK1*), *PARK7*, *PARK8* (*LRRK2*), *PARK9* (*ATP13A2*), *PARK11*, *PARK13* (*HTRA2*), *PARK14* (*PLA2G6*), *PARK15* (*FBXO7*), *PARK17* (*VPS35*), and *PARK18* (*EIF4G1*). Genetic disruption of some these genes has led to the development of additional fly models of Parkinson's disease, recapitulating in flies several aspects of the human disease, including an altered walking gate, death of neuronal cells (such as in the fly eye), and early mortality.

Insights gained from these studies continued to point researchers in the direction of the cell's ability to clean up a mess, which include not only the ability to recycle individual proteins but also the ability to identify organelles that have undergone damage, and recycle them for parts. Mitochondria are the cell's power plants, making energy in the form of adenosine triphosphate (ATP). They exist in cells wrapped in a double-layer of membranes, and have the ability to fuse together or split apart, to stretch thin, and to make more of themselves when needed. One of the insights *Drosophila* research has provided to the Parkinson's disease field was to uncover the idea that both the *parkin* gene product and the *Drosophila* ortholog of another Parkinson's-associated gene, *PINK1*, are required for a process called "mitophagy," the detection, cleanup, and recycling of parts from damaged mitochondria (Clark et al. 2006; Park, Lee, and Chung 2009; Park et al. 2006; Yang et al. 2006). The relevance of this process was confirmed in studies done directly with mammalian cells, including with human iPSCs derived from patients with Parkinson's disease (Hsieh et al. 2016). Research performed in the fly also helped to provide insights into the detailed cellular mechanisms of toxicity by alpha-synuclein, pinpointing where the aggregated proteins clog up the cellular system (Cooper et al. 2006). Similarly, for Huntington's disease, research in the fly suggests a role in autophagy (Rui et al. 2015; Gelman, Rawet-Slobodkin, and Elazar 2015), another clean-up crew process that involves many of the same genes and events as mitophagy. In mitophagy or autophagy, the damaged organelles or other cellular debris are first enclosed in a membrane. This newly formed bag of trash then fuses with an organelle called the lysosome in which the proteins, lipids, and other molecules that make up the enclosed material are broken down into component parts that can either be expelled from the cells or reused to form new large biomolecules.

Now that we can reasonably propose that mitophagy and autophagy underlie cellular events associated with Parkinson's disease, we are able to

ask whether shutting down or amping up mitophagy or autophagy, such as using chemical inhibitors or agonists, makes outcomes worse or better in genetic models of the disease. The results of such studies might point to new routes for therapeutic treatment. Fly researchers are also using fly models of neurodegenerative disease in genetic screens, searching for genetic mutations that worsen or ameliorate disease-related phenotypes in the flies, with assays ranging from detailed cellular analyses using microscopes to the climbing assay or observation of wing posture or flight, additional indicators of the health and activity of nerve-to-muscle connections. Since the development of fly models of neurodegenerative disease in the early 2000s, there have been more than 20 such genetic modifier screens (Lenz et al. 2013). The results of these studies expand the list of genes we can explore in other studies of disease pathology and normal function, not only in flies but also in mammalian models of neurodegenerative disease.

With a growing understanding of the cellular pathology of Parkinson's and other neurodegenerative diseases, researchers are using the fly to ask about earlier events (Chouhan et al. 2016). What are the initiating problems that start cells down a path to neurodegeneration? What are the risk factors? What might the early diagnostic signs be? A number of theories exist regarding the specific catalyzing disruption of the system that messes things up, including accumulation of metal ions such as zinc (Bush 2013; Jellinger 2013) and responses to infection (Maheshwari and Eslick 2015; Kumar et al. 2016; Soscia et al. 2010), which might subsequently lead to increases in levels of proteins such as alpha-synuclein (in Parkinson's disease), amyloid beta (in Alzheimer's disease), or Tau (in tauopathies). For biologists and physician-scientists, at least, there remain unsatisfying ambiguity and uncertainty. We dig deeper. We look for the underlying causes of protein aggregation. We ask if aggregation is a protective response of the cells, contributes to pathology, or both. And, at the whole-animal level, we ask just how early in development or adulthood we can first detect signs of abnormality, such as through testing of learning and memory, neuromuscular control, sensations such as smell, and other neurological functions and behaviors, with the assumption that these early signs will help us better understand the mechanisms of disease.

Ways to Model Neurological (or Other) Diseases

Drosophila research has furthered our understanding of the molecular mechanisms of neurodegenerative diseases. Can this approach to discovery

be applied to other neurological diseases? The answer is yes. Among the things that can differ is the appropriate way in which to model aspects of the disease. As for Parkinson's disease, many fly models of disease are based on genetic manipulation. This can involve introduction of a human gene into flies, as was true for the alpha-synuclein fly models of Parkinson's disease, as well as for the first fly models of neurodegenerative diseases, models of Huntington's disease and spinocerebellar ataxia 3 (Warrick et al. 1998; Jackson et al. 1998). Alternatively, the best approach might be to disrupt the endogenous fly ortholog (when there is one) or introduce specific changes into the fly gene that parallel those found in affected patients. Genetic changes are by no means the only approach to creating fly models of neurological or other diseases. We can model some diseases through injury or mechanical intervention, or alter the environment experienced by the flies, such as by disrupting their sleep cycles or exposing them to toxicants, as in treatment of flies with manganese, rotenone, or paraquat to induce Parkinson's disease-like effects (Bonilla-Ramirez, Jimenez-Del-Rio, and Velez-Pardo 2011; Coulom and Birman 2004; Hisahara and Shimohama 2010). In some cases, even normal behaviors of a fly can be used to model aspects of a disease, which can then be explored further using genetic and other experimental approaches. Moreover, these approaches are not exclusive. The combined effects of genetic disruption and altered sleep might be explored (Chakravarti, Moscato, and Kayser 2017), or a study might seek to model the combined effects of genetics, environment, and aging on development or progression of a disease (Burke et al. 2017).

Below are examples of *Drosophila* models of neurological diseases, or aspects thereof, based on injury, intervention, or observation of relevant normal behaviors. The section that follows explores how, once established, *Drosophila* models of disease can be used in biomedical studies, including for diagnosis and therapeutic discovery.

Mechanical Model of Spinal Cord Injury

Axons are projections that extend from the main cell body of a nerve cell, allowing nerve impulses to travel relatively long distances at rapid speed, and facilitating the kind of reactions that keep a fly out of danger from a predator or keep our legs churning in a running race. A particularly long nerve axon extends along the top of the fly wing, where it helps control flight. When this axon is cut, severing the extension from the main body of

the nerve cell, the axon degenerates, following a stereotyped sequence of cellular events. Researchers can sever the wing axon from the main cell body in different genotypes of flies and look for mutations that result in preservation, rather than degeneration, following injury to the axon. Identifying such genes might help us understand how to preserve human axons that are disconnected from their cell bodies as a result of axon-damaging diseases or spinal cord injury (Fang and Bonini 2015; Soares, Parisi, and Bonini 2014). A different mechanical disruption is used to mimic effects of another neurological injury for which new information would be welcome—traumatic brain injury or TBI. To create a fly model of TBI, researchers attach a vial of flies to a spring, then apply single or multiple physical strikes to the flies in the vial by bending and releasing the spring (Katzenberger et al. 2015; Katzenberger et al. 2013). Here again, the model lets us test the relative responses of different genotypes of flies, as well as identify what genes are expressed or suppressed following injury.

Intervention Model of Depression

One aspect of depression modeled in mammals such as the mouse is the phenomenon of "learned helplessness," a failure to escape or avoid a negative stimulus following stress (Vollmayr and Gass 2013; Seligman and Maier 1967). A learned helplessness assay typically compares mice in each of three groups: animals not subjected to a stress; animals subjected to a stress but maintaining some level of control or ability to escape the stress; and animals subjected to a stress over which they have no control and no means of escape. Within a normal population, animals in the third group will later exhibit signs of "depression" such as decreased movement, passive response to a stress, and failure to try to escape a stress under conditions in which animals from the other two treatment groups would do so. A similar approach has been applied in *Drosophila*. For fly studies, researchers apply a heat stress to a fly only when it stops walking (control group), or apply the heat stress the same number of times but at random with regard to what the fly is doing when the stress is applied (learned helplessness group) (Vollmayr and Gass 2013; Brown et al. 1996). The differences in behavior following the stress trial can then be evaluated and expressed empirically, such as "percent active flies" following exposure to the control or experimental stress conditions. The control and experimental values can be compared to one another to detect the degree of "depression" in the flies exposed to

conditions associated with learned helplessness, and values obtained for different mutant phenotypes can also be compared.

Behavior Model of Anxiety

As introduced in the previous chapter, flies will naturally spend more time near the walls of a chamber than in the middle, a behavior that presumably reduces the risk of predation in the wild. This "wall following" behavior can be quantified through video imaging and analysis of the paths the flies take. Under normal conditions, wildtype flies will rarely cross the open, middle area of the test arena. Increasing stress, such as through treatment with heat, increases the degree of wall following (the flies cross the middle even less frequently). By contrast, treating the flies with the anti-anxiety drug diazepam reduces the effect (flies cross the middle more often). F. Mohammad, A. Claridge-Chang, and colleagues have shown that orthologs of genes known to be involved in anxiety in mammals are relevant to wall following in flies, suggesting that flies can be used for further studies relevant to anxiety and anxiety-related disorders (Mohammad et al. 2016). Wall following also presents a challenge for other types of studies—a propensity for wall following could throw off the numbers for monitoring other types of behaviors. To limit the impact of wall following on other assays, one group of researchers has designed chambers for fly behavior analysis that have sloped walls. These vessels induce flies to distribute themselves more evenly, and were designed in a way that still allows for video recording and automated analysis of individual fly behaviors (Simon and Dickinson 2010).

Putting Models to Work

Once we develop genetic or other types of fly models of diseases or disease-related outcomes, there are at least four ways in which we can use the fly models of disease in studies that affect our understanding of diseases and treatments. Fly models of disease can be used to identify candidate "biomarkers" of disease; to implicate additional genes in disease-relevant processes or refine a list of candidate genes obtained in another study; to reveal cellular changes that ultimately result in what patients experience as symptoms; and to serve as a platform for identification of new protein targets of drugs or new candidate drug treatments.

Biomarkers can help physicians diagnose a disease and track disease progression. *Drosophila* can be used in large-scale proteomics, transcriptomics, or metabolomics studies that help determine whether detection of

a given molecule correlates with the disease. When the change in levels of a biomarker occur in humans as well as in flies, such tests might become incorporated into routine tests of blood and urine, or, when appropriate, added to the panel of tests applied to heel-prick blood samples from newborns. Efforts to find early signs of cellular dysfunction in genetic fly models of neurodegeneration—assigning a "unique profile of neurodegenerative pathology" to diseases such as Parkinson's and Alzheimer's (Chouhan et al. 2016)—are among the fly studies that might end up helping identify human biomarkers that assist physicians in diagnosis of neurodegenerative diseases earlier in adulthood or with increased accuracy. The biomarkers themselves might be genes whose expression changes upon development of the disease; proteins (or their enzymatic activities) detectable at higher or lower levels in patients with the disease; or metabolites that accumulate or are depleted in the disease state but not in unaffected individuals. As any one indicator might not have sufficient predictive power, one likely way forward is for physicians to check a broad panel of biomarkers, with the combinatorial output providing a more accurate picture of health or disease than that provided by any one indicator.

Once a disease model is established, we can use genetic modifier screens to expand our understanding, implicating new genes in development or progression of the disease. If we already know something about the genes identified in the modifier screen, the results can immediately inform understanding. When we find genes for which disruption of the gene makes symptoms worse or outright kills the disease model flies, we might return to human patient DNA sequence data to ask whether disruption of equivalent genes is also associated with the disease or its severity in human patients. By contrast, genes for which disruptions make symptoms better might be good candidate targets for the development of new drugs. Genetic studies can also be used to filter out promising candidates from the sometimes long lists of candidates identified in large-scale human genetic studies. In a genome-wide association study of genes related to restless leg syndrome, researchers identified regions of the human genome associated with high risk for the disease, including a region that corresponds to the human gene *BTBD9*. A. Freeman, S. Sanyal, and colleagues generated flies that are mutant for the *Drosophila* ortholog of the gene, also called *BTBD9* (Freeman et al. 2012). Flies lacking *BTBD9* gene activity do not live as long as their wildtype siblings, have disrupted sleep cycles, and have other phenotypes consistent with restless leg syndrome (Freeman et al. 2012). Use of *Drosophila* to further annotate and prioritize gene lists generated in human genome-wide

association studies is thought to be an area of "untapped potential" rich with opportunity (Wangler, Hu, and Shulman 2017).

In addition to implicating new genes, models of disease also allow for deep investigation of cellular and subcellular mechanisms relevant to the disease. For brain studies specifically, *Drosophila* offers advantages. In 1971, M. M. Herman, J. Miquel, and M. Johnson pointed out that the fly brain does not rely on a vasculature or blood vessel system, so that vascular defects will not be a complication to interpretation of neurological studies of the fly as they can be for some other model organisms (Herman, Miquel, and Johnson 1971). The variety of behaviors exhibited by flies and testable using quantitative and automated readouts further contributes to the usefulness of *Drosophila* in neurological studies. For a simple being, the fly is surprisingly complex. Implication of mitophagy in Parkinson's disease provides an example of how studies in the fly can reveal molecular and cellular mechanisms of disease. Another example comes from a study reported in 2014 by M. Maheshwari, N. R. Jana, and colleagues that focused on Huntington's disease. The study, initiated in mice and continued in flies, implicated down-regulation of a gene called *HSF1*, which encodes a transcription factor, in Huntington's disease. Making a connection to *HSF1* led researchers to consider a drug called dexamethasone, already in clinical use as an anti-inflammatory, as a possible treatment (Maheshwari et al. 2014). Sometimes the phenotypes that link a fly model with a human disease are meaningful but not obvious. In the case of one genetic fly model of neurofibromatosis, for example, disruption of the fly ortholog of a relevant human disease-associate gene leads to an increase in time spent by the animals spontaneously grooming themselves. *Drosophila* researchers compare this behavior with symptoms of attention deficit hyperactivity disorder (ADHD), which is frequently associated with the type of inherited neurofibromatosis modeled in the study (King et al. 2016).

Independent of genetic studies, researchers can ask whether treating the disease model flies with drugs, small molecule inhibitors or agonists, natural products, or chemical derivatives related to traditional medicines can ameliorate symptoms. With flies, we can do these studies in whole animals, assessing large numbers of individuals, and using assays that span the full natural lifespan of the organism, adding to confidence in the outcomes. Methods for exposing a fly to a drug include injecting larvae or adults with the drug; soaking larvae in a solution that includes the drug; providing the drug as an aerosol; and, in rare cases (likely due to the labor involved),

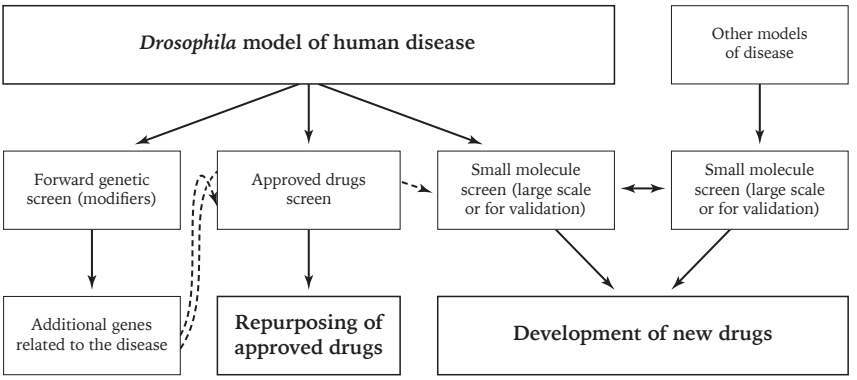
applying the drug to the necks of decapitated flies, which remain alive for a while and can be assayed for drug responses (Pandey and Nichols 2011). The most common method, however, is simply to feed fly larvae or adults with sugar water or food that contains the drug.

An example of the use of *Drosophila* models of disease to identify drug treatments comes from studies of Fragile X syndrome, which is characterized by altered intellectual ability and a number of behavioral disorders, including sleep irregularities and hyperactivity (van Alphen and van Swinderen 2013; van der Voet et al. 2014). The syndrome is associated with disruption of a specific human gene, *FMR1*. Disruption of the fly ortholog *Fmr1* results in changes to neuronal cells that can be quantified and studied, as well as measurable disruptions to fly behaviors, including changes to performance on memory assays and changes in sleep patterns. In common with mouse models of the disease, Fragile X disease model flies also exhibit changes in activity of a specific protein, GluR, that can be targeted with drugs. Chemical inhibitors of GluR were readily available, and testing these inhibitors in flies suggested they might help alleviate symptoms, as when *Fmr1* mutant flies were fed food that contained a GluR-targeting drug, some of the mutant phenotypes associated with the genetic disruption were ameliorated (McBride et al. 2005). Reports in 2009 and 2011 described preliminary but promising results in small clinical trials aimed at investigating the safety and efficacy of GluR-targeting drugs as potential treatments of Fragile X syndrome (Berry-Kravis et al. 2009; Jacquemont et al. 2011).

The 2005 *Drosophila* study by S. M. McBride, T. A. Jongens, and colleagues also provided the first evidence that treatment with lithium, which is already approved for clinical treatment of other conditions and affects GluR-relevant cellular activities, might have a beneficial effect in Fragile X syndrome patients (van der Voet et al. 2014; Berry-Kravis et al. 2008). In 2008, E. Berry-Kravis, W. T. Greenough, and colleagues stated that the results of a small study of human patients were "consistent with results in mouse and fly models," reporting that the treatment is "well-tolerated and provides functional benefits" (Berry-Kravis et al. 2008).

Drosophila in the Drug-Discovery Pipeline

Imagine a news headline: "Tens of thousands of adults treated with unproven, untested, potentially deadly drugs—and not a single person harmed!" It sounds too good to be true until we recognize that the "adults" in question



The use of *Drosophila* models of human disease in a drug-discovery pipeline. Fly models of disease can be used to identify disease-relevant genes or chemical compounds that ameliorate disease-related phenotypes. These become candidates for further testing. For drug screens performed in other organisms or human cells, normal flies can serve as a model for whole-animal toxicity and fly disease models can be used in additional validation steps.

could refer to adult flies. Given the power of the fly—including the ease by which we can test large numbers of individuals and monitor them throughout adulthood—there is interest in both the academic research community and the private sector to use *Drosophila* in drug-discovery pipelines. When applied at large scale, *Drosophila* drug screens can reduce huge collections of small molecules, numbering in the tens or hundreds of thousands, to small subsets of promising candidates that can then be tested in more cost- and labor-intensive animal or human cell models. Large-scale *Drosophila* drug screens are best performed in a highly organized fashion: flies of a specific stage or age, and of a specific genotype, such as an established disease model genotype, are collected and provided control or drug-containing food. Depending on the type and length of the assay, the flies might be provided with fresh food at regular intervals—once a day, every other day, perhaps twice per week. Some researchers perform the tests on flies in standard culture vials; miniaturization can make drug screening more cost-effective and efficient. As few as one to three adult flies can be fed drug-containing food in individual wells of a standard "micro-well plate," a plastic container about the size of a deck of cards made up of 96 individual wells (8 rows by 12 columns), each of which is

essentially a tiny test tube or culture vial, allowing use of a smaller amount of the drug and facilitating a quick and feasible assay readout (Markstein et al. 2014).

As for genetic screening, the drug screen assay is critical to success, and efforts are made to develop increasingly specific, accurate, and automated assays, generating higher quality data and facilitating rapid testing of a large number of drugs in a short period of time. Another consideration in any drug screen is what "library" or set of molecules will be tested for the specific activity of interest. With a large-scale genetic screen, the number of mutagenized flies tested typically numbers in the tens of thousands; with drug screens, the number of compounds tested could easily be in the hundreds of thousands if we wanted it to be. One strategy researchers have used is to limit at least the initial screen to the relatively small number of drugs already approved for use in humans by the FDA. With this approach, the research group might learn information that suggests a drug originally developed to treat one disease could be used as a treatment for another, so that the drug can be "repurposed" in the treatment of an additional ailment or disease (as in the repurposing of lithium for treatment of Fragile X syndrome). Part of the rationale for such as study is that since the drugs being tested in the new context are already in use, the timeline from research study to clinical application can be much shorter than for a study that starts with a new chemical candidate, for which safety, side-effects, dosage, and so on start out as unknowns.

Of course, the validity of any drug screening approach in *Drosophila* aimed at identifying candidates for human disease treatment depends on the assumption that at least some of the candidates identified in *Drosophila* will have similar effects in human cells. This has turned out to be a reasonable assumption. In a search of the literature in 2016, I. Fernandez-Hernandez and colleagues found at least sixty examples of drugs for which a target or mode of action is shared between *Drosophila* and humans (Fernandez-Hernandez et al. 2016). These drugs are not limited to those that work on one type of cellular or protein activity; the drugs target a variety of cellular components and functions, from the cytoskeleton to organelles to signaling pathways (Fernandez-Hernandez et al. 2016). This finding fits well with what we have learned regarding the high degree of conservation of function—of cellular structures, signaling networks, the control of gene expression, cell growth and organ size, neurological activities, and more.

Establishing Cause

Fly models of disease typically start with an established link between a disease and disruption of a specific gene or genes, or perturbation. But for some diseases, we are far behind in our knowledge. We might know or suspect a cause—perhaps the use of a chemical in a given region or profession corresponds with prevalence of a disease, or a human genomic study has identified a new set of candidate disease-related genes—but remain uncertain regarding a true causal link. As discussed in Chapter 2, the wing-spot test provides a quick and quantifiable assay of compounds with mutagenic activity. The battery of behavioral assays available to *Drosophila* researchers allows for testing of neurological effects of suspected toxins or pollutants as well. As also introduced previously, *Drosophila* can help us address the growing need to figure out which specific genes contribute to development or progression of a disease, following the application of genome-wide association studies aimed at identifying candidate disease-associated genes (Shulman 2015; Bellen and Yamamoto 2015).

Figuring out what gene or genes are linked with disease does more than let physicians tick off something other than "unknown" in the diagnosis column. As described, it is a first step toward using the genetic approach and other methods to help identify the molecular mechanisms and can point to existing or new therapeutics. Even when therapeutics are not readily available, a defining cause can have near-term positive impacts on patients, their families, and physicians. Linking a single patient's disease or disorder to other cases, for example, might provide patients and families with the support that comes from shared experience, and allow physicians to give patients and their families more information about what to expect going forward. Below are three examples of how *Drosophila* studies helped reshape our thinking regarding mutations known to be causative for a disease, or helped identify the most promising candidates—or the sole causative gene—from a field of many. For one of the cases presented, information gained in *Drosophila* studies led to the identification of a new rare inherited disorder, Harel-Yoon syndrome, the first to be named (in part) for a *Drosophila* researcher. Although these examples are drawn from the neurological field, the approach is by no means limited to that area. Similar approaches are being applied to other types of diseases.

Rethink the Nature of a Genetic Cause

The various forms of Charcot-Marie-Tooth disease comprise a common inherited neuropathy associated with disruption of one of several known genes (Baets, De Jonghe, and Timmerman 2014). One thing important to our understanding of an inherited disease is to learn whether a given mutation associated with the disease inactivates or reduces function of the gene, or by contrast, increases or changes its function. Given no other evidence, a best guess is that a recessive mutation is likely to be one that inactivates gene function, and a dominant mutation is likely to result in activation or the gain of a function by the gene. But these assumptions do not always hold true. Charcot-Marie-Tooth type 2B (CMT2B) is an example of a disease with a dominant pattern of inheritance, with one altered copy of the human gene *RAB7A* (commonly referred to as *Rab7*) sufficient to result in disease. Based on the pattern of inheritance, protein structure analysis, and other studies, CMT2B-associated mutations in *RAB7A* were thought to be gain-of-function mutations, such that a route to therapy might include identification of drugs that reduce RAB7A protein activity. In 2013, however, S. Cherry, R. P. Hiesinger, and colleagues reported experimental results obtained in *Drosophila* that suggested a need to rethink the gain-of-function hypothesis for CMT2B-associated mutations in *RAB7A*. In experimental analyses that included application of eye-specific genetic manipulation, electron microscopy, and electroretinography, the researchers obtained data suggesting that CMT2B-associated mutations in *RAB7A* are associated with partial loss of function, rather than a gain of function (Cherry et al. 2013).

M. K. Sadanandappa and M. Ramaswami summarized the results and implications at the time the report was published, stating that there is "strong evidence to support the notion that CMT2B disease pathologies arise from the partial loss of Rab7 function. This new work forces a reexamination of the mechanistic basis for CMT2B and suggests that patients may need to have the activity of the Rab7 pathway stimulated rather than reduced" (Sadanandappa and Ramaswami 2013). In a 2015 article coauthored by several members of the advisory board of the Hereditary Neuropathy Foundation, these leaders in the field similarly stated that the fly results have "quite possibly overturned our basic understanding of the disease mechanism behind CMT2B" and that "Perhaps experimental methods to increase Rab7 protein levels, while opposite to the previous dogma, may

actually be correct" (Ekins et al. 2015). They further point out that the *Drosophila* study resulted in the "first animal model" of CMT2B and suggest that the "increasing utilization of different animal models of various CMTs suggests that their role will likely increase in importance and may lead to useful insights and help identify therapeutics" (Ekins et al. 2015).

Identify Multi-Gene Effects

Among the most challenging genetic disorders to approach and understand are those that involve changes in more than one gene. Examples include disorders or syndromes associated with chromosome nondisjunction or duplication events that result in the presence of extra copies of an entire chromosomal region in the genome of affected individuals. The best known of these is Down syndrome, which results from nondisjunction of chromosome 21, such that affected individuals have three copies of genes on that chromosome instead of two. We commonly associate Down syndrome with intellectual disability, but it is associated with additional medical challenges as well. For at least some of these, the effect depends on the specific subregions of chromosome 21 present in three copies. An example is a congenital heart defect present in some but not all Down syndrome cases. By 2009, studies in mice, together with detailed human genetic mapping (Korbel et al. 2009), had limited the chromosomal region implicated in the heart defect to one end of chromosome 21. However, although three likely candidate genes could be identified, there remained uncertainty regarding which specific genes were at fault and the precise relationships among them.

A 2011 study by T. R. Grossman, A. Gamliel, and colleagues cleared the fog. They first tested the effects of overexpressing the mammalian or fly version of any of five candidate genes in the region, using the Gal4-UAS system (Chapter 2) to target the study specifically to the *Drosophila* equivalent of the heart (Appendix B). Three genes scored as positive in at least two assays related to *Drosophila* heart disease. To better mimic what happens in Down syndrome patients—wherein more than one gene is present in an extra copy—the researchers then created flies in which any combination of two of these three genes is expressed at increased levels. They found that increased expression in the fly heart of two genes, *COL6A2* and *DSCAM*, together resulted in a strong effect on fly heart function. They went on to do a similar test in the mouse heart and made similar findings. Although the animal models do not exhibit all the defects observed in the

hearts of Down syndrome patients with congenital heart disease, the findings suggest an avenue worthy of pursuit (Grossman et al. 2011).

Solve a Mystery

The NIH-funded Undiagnosed Diseases Network (UDN) is designed to address the problem that "Every year hundreds of men, women and children face hardships and uncertainty when their healthcare providers are unable to discover the cause for their symptoms" (Undiagnosed Diseases 2017). Next-generation sequencing provides an opportunity for medical genetics experts and others to identify candidate genes that underlie individual cases. In some cases, genome sequence data point clinicians in the direction of previously identified diseases. In others, the data suggest that the disease might be one previously uncharacterized or undefined at the level of genetic cause. The UDN has purposefully turned to *Drosophila* and other model organism studies in order to help identify genetic causes of previously undiagnosed diseases in an efficient and effective manner. In one study, UDN experts identified five individuals that had similar sets of symptoms and had in common the same variant (DNA change) in the human gene *ATAD3A*, as well as two additional families in which the same gene was altered in affected individuals (Harel et al. 2016). Moreover, the clinical data suggested that defects in mitochondrial function might be relevant, suggesting that if *ATAD3A* were indeed the cause, then the normal function of the gene would be related to mitochondria.

To further test the hypothesis that differences in *ATAD3A* cause the genetic disease observed in these families, as well as examine possible cellular mechanisms, researchers turned to *Drosophila*. Flies have a single ortholog of the *ATAD3A* gene, a gene that had been named *belphegor* (*bor*). In the fly study, W. H. Yoon, H. J. Bellen, and colleagues tested the effects of making the specific DNA change found in five families (which results in a change in the 534th amino acid of the encoded human protein from arginine to tryptophan) in the fly version of the gene. They used the Gal4-UAS system to express either wildtype *bor* or the mutant form in neuronal tissue, then looked at the mitochondrial content of neuronal cells. Examining these cells at the subcellular level, they observed a loss of mitochondria. Altogether, the efforts of a group of experts in human and *Drosophila* genetics defined *ATAD3A* as the cause of the disease common to members of the seven identified families. In recognition of the contributions of medical

geneticist T. Harel and *Drosophila* geneticist W. H. Yoon to defining this important functional relationship between *ATAD3A* and the newly identified genetic syndrome, the disease was named Harel-Yoon syndrome (Harel et al. 2016). A similar study linked the gene *EBF3* to a different rare and previously undefined neurological disorder (Chao et al. 2017).

Working Together, Working Toward Cure

In 2009, the pediatrician K. N. Bharucha suggested that fellow pediatricians should befriend colleagues with fly laboratories "as part of the start of a productive collaboration" on disease-related topics (Bharucha 2009). The UDN has formalized a link from clinic to fly lab by funding a UDN-associated model organism screening center that includes a *Drosophila* research component. Similar collaborative efforts that span the intellectual distance from fly-pushing station to clinic have been undertaken at the national level, within and among research institutions, and among individual labs. In some cases, new information gained regarding the cellular mechanisms of disease lead quickly to ideas regarding treatment—such as when a disease is meaningfully linked to a signal transduction pathway or enzymatic activity for which approved drug treatment strategies, or candidate inhibitors and agonists, have already been developed. But this is not always the case. There is as yet no satisfying end, for example, to our travel toward the development of curative treatments for Parkinson's disease, Alzheimer's disease, or other neurodegenerative diseases. Likewise, though a genetic cause and a name have been assigned to Harel-Yoon syndrome and other rare neurological conditions, the insights gained do not necessarily reveal a clear path to treatment. Is this a failure? A reason to abandon *Drosophila* and other animal models of disease? In considering the answer we must keep in mind that thus far, no other research path has led to a cure for Parkinson's disease or other neurodegenerative diseases either. We can seek to balance two human attributes that can contribute to future progress: a willingness to take risks, as is inherent in betting on the fly as a route toward understanding and curing diseases, and a willingness to have patience.

As stated in 2015 by a group of neurological disease experts, when such experts think about animal models of disease "the fruit fly (*Drosophila melanogaster*) is likely the furthest from our mind" (Ekins et al. 2015). Even for relatively informed biological and biomedical researchers, it can

take a leap of faith to imagine that this small insect—so radically distinct from our talking, philosophizing, sophisticated human selves—can reveal something that studies in a more closely related organism might not. But the accumulated data clearly suggest that, given their short life cycle and similarities to humans, including in the area of neurological function and behaviors, *Drosophila* do indeed provide a platform for accelerated study of human diseases: fly research studies meaningfully contribute to our understanding of underlying causes, cellular mechanisms, and potential treatments. When it comes to both common and rare neurological (and other) diseases, improved diagnosis, understanding, and treatments are needed. We can build a rational, results-based argument that what will be learned from further use of *Drosophila* has the potential to lead to both small advances and key breakthroughs, adding up to genuine help as we seek to unlock the secrets of disease. Particularly when *Drosophila* studies are coordinated with efforts carried out in other species—from cellular studies in yeast to medical genomics—these studies have real potential to inform and ultimately, to help relieve the heavy burden of neurological and other diseases on humankind.

CHAPTER TEN

CONTINUITY

THE ABDOMEN OF A NORMAL ADULT FEMALE FLY CONTAINS A PAIR OF ovaries, which look at first glance like two clusters of bananas connected to one another at their stem ends. When the ovaries are removed and a single elongated unit—an "ovariole"—is teased away from the others, we can observe a further sub-organization. Each ovariole is itself made up of a string of football-shaped "egg chambers," connected to one another by "stalk" cells. Egg chambers at the anterior end of the ovariole are small and rounded, whereas the more posterior egg chambers begin to take on the shape of a mature *Drosophila* egg, growing larger and more oblong. Nestled at the extreme anterior tip of the ovariole, we find a small number of specialized cells called "germline stem cells" (GSCs) that have the unique ability to divide, mature, and become the eggs, contributing to the next generation. A similar setup is present in a male fly. Male GSCs, which will divide and form spermatozoa, are nestled at the anterior ends of the testes—another pair of visually striking structures, each one curled like a fiddlehead fern and, along most of its length, filled with the wavy curves of sperm tails.

What would fly researchers discover at these extreme tips of the reproductive structures? In the immediate environment in which the GSCs sit? Shangri-La.

Life and Death

In the nineteenth century, embryologist A. Weismann proposed that death is not inevitable; rather, it is an adaptation of being a multicellular organism. He reasoned that for single-celled organisms, which "increase by division," growth is "not connected with the death of the old." Death arose in multicellular organisms, Weismann argued, as they became increasingly complex, with so many distinct specialized cell types that "an unending life became disadvantageous," with the body "a secondary appendage of the real bearer of life—the reproductive cells" (Weismann 1889). Of course, the body could in theory persist long after it had concluded its role in continuation of the species. But Nature is cruelly efficient, and, as Weismann stated, "an unlimited persistence of the individual would be a luxury without a purpose." In a sense, then, the GSCs are the only cells in the body with some semblance of immortality. Once the fly reaches adulthood, these cells form the eggs or sperm that together contribute to an embryo, such that the GSCs—or at least, half portions of their DNA—live on.

But life springs from a source. If the GSCs at the far anterior end of an ovariole or testis of a young adult fly form only eggs and sperm, then the store of GSCs would quickly be depleted, and the fly rendered sterile. The solution to this problem can be likened to the person who uses one of two wishes to wish for two more wishes. If GSCs divide to form one cell that goes on to be egg or sperm, and another that stays a GSC, then production of new eggs or sperm can go on indefinitely. This seems straightforward enough, but raises another question. When a stem cell divides, each daughter cell is presumably the same as the other—like twins. So we have to ask, What induces one of the two daughter cells to make an egg or sperm, and the other to remain a stem cell?

Defining Paradise

We humans routinely lose blood cells—through natural death in the bloodstream, through menstruation, or through trauma such as cuts, bruises, or blood blisters. But we don't run out of blood. We make more. At least by the 1960s, experts in the field of mammalian "hematopoiesis," or blood cell formation, knew that this ability to make more blood cells was a special property of cells that are normally found in the bone marrow and a few other locations. The existence of these specialized "hematopoietic stem cells" was evident in the ability to restore blood formation in irradiated

mice or rats by transplanting a source of these cells from an untreated animal into an irradiated one (Ford and Micklem 1963; Ford et al. 1956). Moreover, researchers garnered from these studies that the site of the transplant was not exacting. Blood precursor cells will "home" to specific locations such as the bone marrow, spleen, or thymus; take up residence there; and only then proliferate, generating new blood cells. When the same type of cell gets stuck in a different location, such as the lung, the cells do not exhibit the same behavior—that is, there appeared to be something special about "reaching home" that entices the cells to stay, proliferate, and generate new blood cells (Trentin 1970).

The experimental biologist J. J. Trentin proposed in 1970 that within the bone marrow and other home locations, there exists a "hematopoietic inductive microenvironment" with the unique ability to serve as a home location for blood stem cells. In the later 1970s, another blood cell expert, R. Schofield, referred to this specialized microenvironment as a "niche" (Schofield 1978), introducing the term that would stick and eventually, become widely applied to describe the microenvironment surrounding any type of stem cell. Fly biologist H. Lin describes a stem cell niche as "the Shangri-La, the idyllic hideaway" in which these cells reside (Lin 2002). Nestled in the niche, Lin states, stem cells "thrive to self-renew and to produce numerous daughter cells that will differentiate and age as they leave the paradise" (Lin 2002). In other words, the niche is the place that a stem cell is granted its two wishes—allowing it both to remain and to become something else.

Though the *Drosophila* ovary is a structure with a different purpose—one designed to make fly eggs, not blood cells—the ovary, and specifically, the substructure of the ovary known as the "germarium," have similarities with what was described for hematopoiesis. The organization of the cells that make up the germarium can be likened to a knitted winter hat pulled over the wearer's head. The extreme end—the pompon—comprises a set of cells called the "terminal filament" cells; the top of the hat is formed by anterior "cap" cells; and the sides of the hat are made up of more lateral "inner sheath" cells that surround the GSCs themselves. Within this small cluster of cells, precise positions matter. When a GSC divides in two, one cell remains in contact with the cap cells and the other loses this immediate proximity. The cell that remains in contact with cap cells remains a GSC. The more posterior-positioned cell, by contrast, goes on to form an egg. A similar setup exists in the *Drosophila* testes, in which "hub" cells sit immediately anterior to the male GSCs (Kiger et al. 2001; Tulina and Matunis

2001). A question arises, what is special about the proximity of a GSC to a cap cell that keeps one daughter of a recently divided stem cell a stem cell while its sister goes on to form an egg?

The study of stem cell niches in mammalian systems presents an "arduous endeavor" (Lin 2002); in comparison, the fly germarium is relatively easy to manipulate. In the 1990s, H. Lin, A. C. Spradling, and others used of a number of approaches to study *Drosophila* GSCs and their niche, including killing specific cells in the germarium with precisely directed lasers; transplantation of cells from the ovary of one fly to another; and genetic perturbations that included the dialing up or down of Hh pathway signaling (Lin and Spradling 1993; Forbes et al. 1996). The researchers found that following laser ablation of cells surrounding the GSCs—that is, killing the niche cells—all the GSCs went on to form eggs, and the system was quickly depleted of its GSC reserve. Moreover, through genetic analyses the researchers identified specific genes required in the niche cells to maintain GSCs within the niche, as would be deduced for a gene that, when disrupted in niche cells, has the same effect as laser ablation of those cells. These studies are credited with providing the first clear experimental evidence of a stem cell niche, as well as defining what genes—what signaling pathways and other cellular activities—are important to the process. Many of the same pathways relevant in other cell types proved relevant to communication between the niche and GSCs, including the Hh pathway (Lin 2002). The genes required for suppression of transposon mobilization by the piRNA system (Chapter 7) also have relevance to the GSC niche (Szakmary et al. 2005; Jin, Flynt, and Lai 2013); disruption of the *piwi* gene, for example, can lead to uncontrolled proliferation of GSCs.

As a result of extensive molecular genetic studies of the relationship between the niche and GSCs in the *Drosophila* ovary and testes, we have an increasingly detailed understanding of how GSCs remain in the niche, what signals are sent from niche cells to the GSCs, and how those signals affect the decision to become a stem cell or go on to form an egg or sperm (Greenspan, de Cuevas, and Matunis 2015). As for many other biological events, much of what was uncovered in the fly in terms of genes and pathways turned out to be relevant to what goes on in stem cell niches in other animals as well. Transplantation experiments in 1994 demonstrated the existence of a stem cell niche in the mouse that supports GSCs (Brinster and Zimmermann 1994), and the ability to reconstitute the male mouse GSC system in vitro, as reported in 2012, provides new routes to experimentation

(Kanatsu-Shinohara et al. 2012). Moreover, although more challenging than in the fly, in the mouse it is possible to manipulate gene function in a tissue-specific manner. Applying this approach, researchers found that the organization of the mammalian hematopoietic stem cell niche, as well as the molecular mechanisms by which hematopoietic niche cells communicate and interact with the stem cells, have commonalities with what was found for GSC niches in the fly (Merchant et al. 2010).

Continuity within the Adult

The relevance of the stem cell niche, and stem cell functions more generally, is not limited to blood, eggs, and sperm. Many cell types are in danger of cell loss or damage, and thus require a mechanism for self-renewal—an active system that maintains "homeostasis" or sameness in the face of challenges. The skin might be cut or abraded or the gut stretched to its limits by the intake of food, making the organism's very survival dependent upon repair. A reserve of skin, gut, and other types of stem cells provides these tissues with an at-the-ready resource—these stem cells can divide to form new skin or gut cells, while at the same time retaining a population of stem cells within a niche. We can appreciate that such a process must be carefully regulated; we do not wish our skin to grow five times thicker following a sunburn or our gut to become blocked by the uncontrolled division of its own cells. Thus, the process of self-renewal is limited to stem cells and division of these cells is tightly controlled. The *Drosophila* ovary, testes, gut, and nervous system have all emerged as useful experimental settings in which to explore stem cells and tissue homeostasis (Homem and Knoblich 2012; Jiang and Edgar 2011; Greenspan, de Cuevas, and Matunis 2015). As we begin to get a grasp on what happens in any given stem cell niche, we can appreciate, too, that activities at any one location are likely coordinated with what is going on elsewhere in the organism. L. E. O'Brien and D. Bilder pointed out in 2013 that within a tissue such as the gut, there are several clusters of niche and stem cells, and that in addition to each regulating themselves, there is a need, also, for coordination across the whole organ. "The next challenge in stem cell biology," these authors suggested, "is to understand the higher order processes that coordinate stem cell-niche units at the whole-organ level" (O'Brien and Bilder 2013).

Another area of interest is to explore what happens to stem cells and tissue homeostasis—to the maintenance process—as we age. In studies re-

ported in 2011 and 2012, M. Rera, D. W. Walker, and colleagues added blue dye to fly food and then asked whether that blue color is restricted to the simple tube of the gut running through the adult, as is visible in the translucent belly of the fly, or whether the dye turns the whole fly blue, indicating that the gut has become permeable (Rera et al. 2011; Rera, Clark, and Walker 2012). This assay, known as the Smurf assay (Rera, Clark, and Walker 2012), gives a quick, high-level indication of gut damage that can be followed by more detailed analyses such as detection of stem cell division and signal transduction pathway activity in dissected fly guts, or electron micrograph imaging of the mucus layer that separates the food from the gut cells. An increased prevalence of gut permeability turns out to be associated with aging in flies, and might be one of a cluster of events associated with a late-in-life "death spiral" (Mueller et al. 2016). Moreover, "Smurfing" (turning blue in the assay) has been observed not only following direct perturbation of the fly gut but also among flies subjected to brain trauma, suggesting that gut permeability might be a general hallmark of impending death (Katzenberger, Ganetzky, and Wassarman 2015). The Smurf assay—which requires no fancy equipment, no dissection, no costly reagents—has since been used in studies of two other *Drosophila* species (*D. mojavensis* and *D. virilis*), as well as in worms and zebrafish (Dambroise et al. 2016). New assays allow us to ask new questions; the answers to those questions lead us forward to the next.

Continuity across Generations

As uncovered in part through the work of the early Fly Room group, our chromosomes—our DNA—provides continuity in the form of information. Traits, behaviors, and activities are encoded in the genome and passed from parent to child. One thing biologists have not thought of as inheritable (in the genetic sense of the word) from our parents or grandparents is personal experience. As humans, we use language and reasoning to learn from the stories and hardships of others. Ideas "go viral" and spread as "memes" through communication. But the commonly accepted notion has been that this type of information, our experiences of life, good or bad, do not become written into the genes; they do not alter the genetic code as we experience them. Instead, evolution edits the sentences of life slowly: populations can evolve over time and there is no way for individuals to adjust, to make quick heritable changes in response to some environmental stress that might be likely to affect the offspring as it has the parent.

Though largely true, the idea that individual experience is not passed along in the DNA is not without exception. We are finding out, through work in several species, including *Drosophila,* that there are ways for organisms to make last-minute changes that are heritable—changes referred to as "transgenerational" effects. These heritable changes do not result from direct base-pair sequence changes to DNA, but they do alter DNA, coming in the form of epigenetic marks on the chromosomes, as introduced in Chapter 6, that affect what genes are turned off or on. Various stresses can induce changes that influence what genes are turned off or on in offspring of the affected individuals. There are indications, for example, that the experience of starvation or obesity by a parent can change what epigenetic marks are present on specific subsets of genes. When those marks are present in GSCs, the effect can be inherited, influencing the level of expression of specific sets of genes in offspring and resulting in changes to physiology, immunity, or other processes relevant to the initial stress. Despite the fact that these effects impact our fundamental understanding of inheritance and can have human health implications, in 2017, F. Ciabrelli, G. Cavalli, and colleagues noted that "very little is known about the principles and the molecular mechanisms governing this type of inheritance" (Ciabrelli et al. 2017).

Studying transgenerational effects presents specific challenges. The difficulties of performing genetic analyses with an organism that has a long lifecycle are compounded in transgenerational studies, wherein the phenotype we are looking for will manifest one or more generations after the stress or other treatment is applied. Moreover, the phenotypes observed might be subtle and more variable than are the effects of true mutational changes to the DNA sequence, requiring observation of many more animals to gain confidence in an observed effect. Thus, *Drosophila,* with its short life cycle and ability to reproduce in large numbers, facilitates study in this area. Researchers have found evidence of transgenerational effects in response to changes to the fly diet, and are testing the influence of specific genotypes on these effects to better understand what factors are relevant (Dew-Budd, Jarnigan, and Reed 2016; Somer and Thummel 2014; Brookheart and Duncan 2016a; 2016b). Those interested in specific mechanisms of some transgenerational effects, such as the relevance of Polycomb proteins (Chapter 6), have developed specially engineered fly strains they call "epilines" useful for these studies (Ciabrelli et al. 2017). Moreover, in the field of immunity, new discoveries in transgenerational effects and related

areas have brought on a rethinking of long-held ideas regarding the capacity of an insect to respond to specific threats and protect their offspring from the threat (Pigeault et al. 2016; Tidbury, Pedersen, and Boots 2011). Rather than being naïve to any new threat, exposure of insects to some pathogens appears to bring about changes that make the response of the same insect to a next infection more robust (within-generation "priming") and confer increased protection to offspring (transgenerational priming). In this area, as in others, what is learned in the fly system is being integrated with what is learned from studies of other species, with the synergy of a cross-species approach pushing new fields forward at a fast pace.

The Rate of Living and the (Dis)Continuity of Ideas

Even with stem cells in place to maintain homeostasis, and given the various endowments we inherit from our parents, we nonetheless face the seemingly inevitable decline of age and ultimately, death. Aging was among the topics that fascinated the researcher R. Pearl in the first half of the twentieth century. Pearl was an early proponent in the United States of biostatistics and big data science; he was interested in compiling data at the population level. Accordingly, when Pearl decided to focus his attention on age-related studies rather than individual cases, he was interested in what we would now refer to as average lifespan across a population, a measure he referred to as the "duration of life" (Pearl 1928). Before undertaking work in this area, Pearl sought an organism suited to this type of study. The criteria he wished for were "(1) that the animal should be short-lived so that mortality statistics would accrue with reasonable rapidity; (2) that it should breed freely in captivity, so that the statistics of natality should not lag behind those of mortality; (3) that its husbandry in the laboratory should be relatively simple; . . . and finally (4) that it be a form whose genetics was fairly well known" (Pearl 1928). Sound familiar? After dallying with mice for a while, in 1919 Pearl settled on *Drosophila* for his studies in this area. "Up to the time when our work began," he stated later, "little had ever been done in any systematic way on the experimental study of the normal duration of life. Curiously enough most of this had made use of *Drosophila* as material" (Pearl 1928). Pearl noted a 1913 study by R. R. Hyde on inheritance of longevity, in which Hyde describes a strain of flies with a shortened lifespan, but criticized Hyde's studies, stating that "his numbers were too small" (Pearl 1928). In one of Pearl's subsequent studies, 10,000 flies

might be tracked from birth to death in a single experiment. Power in numbers.

One of the impacts of Pearl's studies with *Drosophila* was that they provided clear evidence, backed by large numbers, that lifespan is under genetic control; that our genes influence how long we are likely to live (Pearl 1922; 1928). We now take this idea for granted. Ongoing studies, for example, are examining the genomes of centenarians—persons who live to be 100 years or more—looking for clues in their genomes as to what genetic differences contribute to the ability of one person to live to be a hundred. In the *Drosophila* field, researchers have found genes for which mutations result in short or long-lived flies, and are beginning to understand the molecular mechanisms that allow for lifespan extension, which include relationships to signal transduction pathways, transcription factors, and other types of molecules. Fly research has even begun to point to possible strategies for lifespan extension, such as by targeting activity of the insulin signaling pathway using the drug trametinib, which, as demonstrated by L. Partridge and colleagues in 2015, can extend lifespan in the fly (Slack et al. 2015).

Among other ideas Pearl offered up was a theory, for which he was arguably best known in his lifetime, that the rate of aging of an organism is related to its "rate of living" or metabolism (Pearl 1928). Even if we were to credit this as a first-in-fly concept—which, given that Pearl's work in this area extended to other species, including cantaloupes, is a difficult case to make—that credit would not be worth much. Though influential in its day, the rate of living hypothesis has since been discredited. By contrast, some concepts and ideas put forward by Pearl that were not well accepted in his time were later validated and accepted. For one thing, he is credited by some as the first to suggest that moderate drinking of alcohol has a health benefit as compared with either abstinence or excess, and that smoking is bad for our health (Goldman 2002; Pearl 1924a; 1924b). Many rejected these ideas at the time, and neither had general impact on public health recommendations for a long time after Pearl's proposal. For both ideas, however, further accumulation of population-level data, as well as other types of evidence, would end up lending support. The ideas that moderate drinking can be good for health and that smoking is bad were eventually accepted, influencing health policy worldwide.

Continuity in Pursuit

Pearl's work on the "rate of living" and on the health effects of alcohol or smoking, together with their eventual debunking or acceptance, exemplifies the long arc a research path can follow. Decades can separate the proposal of a scientific idea or hypothesis from the point at which sufficient evidence has accumulated to reliably reject or accept it. Moreover, as we continue to explore, in many cases what once seemed clear becomes fuzzy again, and fields of study that seemed only distantly related converge. Through research initiated in *Drosophila,* we have come to a new understanding of just how connected we all are by common genes, signals, pathways, complexes, and mechanisms, and by the path of evolution. Genes do not act alone or only once, but act in networks and iteratively, in multiple life stages, tissues, and organs. Solutions are not applied once, but over and over, reutilized in new contexts during development and across evolutionary time. Organisms do not exist in some sterile separated world, but interact with one another and with symbionts, parasites, and predators. Individuals, and collectively, species persist over time through reproduction, with each generation both a reflection of and a divergence from the one that came before.

Ultimately, to truly understand an organism—to understand health, aging, and death, even in this one tiny fly, a simple proxy of our more complex selves—we need much more information than we have now. We need to understand every event from conception to the ultimate demise; understand the full life history of an individual; have a complete picture of anatomy, physiology, and metabolism. We need to know the separate and integrated roles of chromosomes, genes, RNA, proteins, signals, cells, organs—both as the organism forms during development and as it changes with age. And what better system than *Drosophila* in which to try to achieve this—first? In which to seek a fully comprehensive picture of a complex multicellular creature, to know gene by gene, cell by cell, and moment by moment, what happens over the full course of a complex multicellular organism's lifetime? To inventory all the parts and read the entire manual, the whole story, with all its footnotes, appendices, and alternate endings?

We cannot know all that such an exploration will teach us. But we can reasonably predict that we will learn new information from such an endeavor. Not from every experiment; not from every screen, assay, or analysis. But from very many of them. In the words of C. Stern, "progress often

proceeds best on the basis of past accomplishments. New questions may be asked on the basis of old experiments and sometimes answers are possible because of information already available" (Stern 1954). The effort to gain a complete understanding of this one species is, of course, an effort already more than a century underway, with each *Drosophila* researcher making some contribution, large or small, and each new technical advance speeding things along. As we continue, we will uncover both exciting new truths and incremental findings. From the whole, we gain deeper insight into this one remarkable creature, this one tiny fly known as *Drosophila melanogaster,* and at the same time, gain insight into so many other living creatures—other insects, other animals, plants, fungi, microbes. And ourselves.

EPILOGUE

AS EMBRYOLOGIST E. G. CONKLIN WROTE IN 1929, "ALMOST EVERY BIOlogical problem can be approached from many sides, and in its final solution many methods are needed" (Conklin 1929). *Drosophila* research is at its most valuable when considered as a part of a larger general effort with the shared goal of uncovering information about living systems, including which genes and gene functions are the same and which are different among different organisms. I do not know of any researcher who reads only about *Drosophila* or another single species. That kind of intellectual isolation would kill a career. Instead of being myopically focused on one species—even one as fascinating as *Drosophila*—we are each aware of, borrowing from, and adding to what has been learned using a variety of species; each contributing a few drops of water to the collective rush of new knowledge that advances progress. Related to this, in addition to identifying ourselves as "fly people," many of us working with *Drosophila* also consider ourselves part of other research subgroups. We might define ourselves as researchers in biochemistry, cell biology, developmental biology, genetics, ecology, entomology, evolutionary biology, neuroscience, physiology, population biology, and so on, with the group identity defined by the topic of study, rather than by the experimental organism used to investigate that topic.

In addition to emphasizing the vital connection between *Drosophila* research and studies in other systems, I also want to emphasize that the selection of "firsts" I presented here are in no way the be-all, end-all of *Drosophila* research. For one thing, I freely admit that "firsts" are not easy to define; many discoveries are mired by oversight, competition, or misattribution. For another, "firsts" should not be a sole or even a principle measure of success, either within a field or outside of it. As G. M. Rubin stated in 2015, "Our fortunes rise and fall together, based not on which of us publishes some result first, but on how the value of fly research as a whole is viewed by others" (Rubin 2015). And what is that value, as we view fly research more than one hundred years (and 100,000 research papers) after its start? I am biased, of course, but in examining the evidence, I feel we cannot help but be won over to the lasting impact and continued potential of *Drosophila* research, including but not limited to connections between what we are learning from the fly and our understanding of human biology, health, and disease.

Moreover, though I have focused for the most part on what we do know about *Drosophila,* a multitude of mysteries remain. Even the *white* gene still holds surprises; subsequent to the early twentieth-century identification of the gene as required for eye pigmentation, additional roles for *white* continue to be uncovered, including a role in male courtship behavior independent of vision (Lee et al. 2008; Krstic, Boll, and Noll 2013; Anaka et al. 2008; Zhang and Odenwald 1995) and a role in the development of insulin resistance in response to a high-sugar diet (Navrotskaya et al. 2016). Similarly, the functions of "famous" signaling pathways like the Hh, Toll/TLR, and PCP pathways are not yet fully understood. New roles are still being uncovered, with new genes added to the long lists of interactors with previously identified components of the pathways, and new connections found among pathways, suggesting levels of interdependence and interaction we are only beginning to detect, understand, and appreciate (Baena-Lopez, Nojima, and Vincent 2012; Grusche, Richardson, and Harvey 2010; Doroquez and Rebay 2006). In addition, we are still actively developing new phenotypic assays—new tests—and new human disease model fly strains that can be combined with genetic screens, drug screens, and other methods to uncover new information.

With each newly developed test or method, and indeed simply given technological improvements that allow us to repeat with more accuracy the same tests we have already done, we increase the probability of defining

even more roles for more genes, learning more about both the genes already well known to us and the perhaps surprisingly large number of *Drosophila* (and human) genes that remain annotated in databases as "function unassigned." Relatively recent findings such as the post-genome elucidation of the Hippo signaling pathway; the co-discovery in flies and other organisms of a new cellular structure called the "cytoophidium" or cell snake (Liu 2010; Noree et al. 2010; Ingerson-Mahar et al. 2010; Aughey and Liu 2015); the finding, using flies, that zinc plays a role in formation of kidney stones (Chi et al. 2015); and the identification of a fly protein equivalent to human leptin (Rajan and Perrimon 2012), which was previously thought to be absent from insects, continue to rewrite biological and biomedical textbooks. There remain many areas for which contemporary experts describe our understanding as limited, incipient, lacking mechanistic understanding, or even "rudimentary, at best" (Hariharan 2015), as was said in 2015 regarding our understanding of the control of organismal size—despite what had been elucidated by then regarding Hippo signaling.

Altogether, no reasonably informed person could argue that we know all there is to know about *Drosophila melanogaster* or that we have already learned all it can offer that is relevant to understanding other organisms. Instead of concluding that a hundred years of research has fully illuminated the fly, the lesson should be the opposite: that one hundred-plus years full of surprises and insights should convince us to push forward, to continue our investment in fly research and put this exceptional system to use for another hundred years or more. At that point we might still not have exhausted the usefulness of the system, for example, in areas that are just beginning to be explored to their fullest. These include the identification or testing of potential therapeutic drugs using whole-animal models of human diseases, holding perhaps particular near-term promise in the areas of cancer, immune functions, and neurodegenerative diseases; the influence of age and diet on health-related measures such as lifespan or recovery following injury; the genetics of interspecies interactions, such as studies related to parasitic wasps or the gut microbiome; and the impact of space travel or other extreme conditions on a living system. In each of these cases, *Drosophila* provides a convenient and already well-understood system for further study.

In addition, the story of *Drosophila* research is one that demonstrates the tremendous value of curiosity-driven research. Many original lines of

study that ended up having an impact on biomedicine did not begin with human diseases foremost in mind—or even in remote consideration. They began instead with curiosity and interest, a desire to uncover a truth, a drive to get an answer to the question "How does that work?" for some biological process that happened to fascinate the researcher. Connections with human health revealed themselves later. It follows, then, that even if we were to decide as a society that the value of biological research lies solely in the ability of research findings on understanding of human health and development of treatments for disease, rather than placing value in new knowledge for its own sake, we would still support fundamental, curiosity-driven studies using a diverse set of organisms, even when connections to disease are not yet apparent. If we limit what topics are studied, or what model systems are used in those studies, we risk losing the chance to make new and nonobvious connections in the future.

More than twenty years after getting started in the *Drosophila* research field, and though I have witnessed a number of revolutions in biology—from the completion of the Human Genome Project to the rapid development of the CRISPR system as a genetic research tool—I remain a "fly person," as intrigued and committed as ever. Biological research has lasting human interest, value, and impact. And happily for those of us in the field, biological research is, every once in a while, incredibly exciting. My best hope is that this small taste of what has been learned from research in just one model system will encourage you to voice support for model organism research and, for at least a few of you, that it might entice you to join our ranks, applying the genetic approach and other methods to the study of some question that intrigues you, using whatever experimental organism or organisms best fit the question.

Despite all we have learned, there remains so much more to uncover.

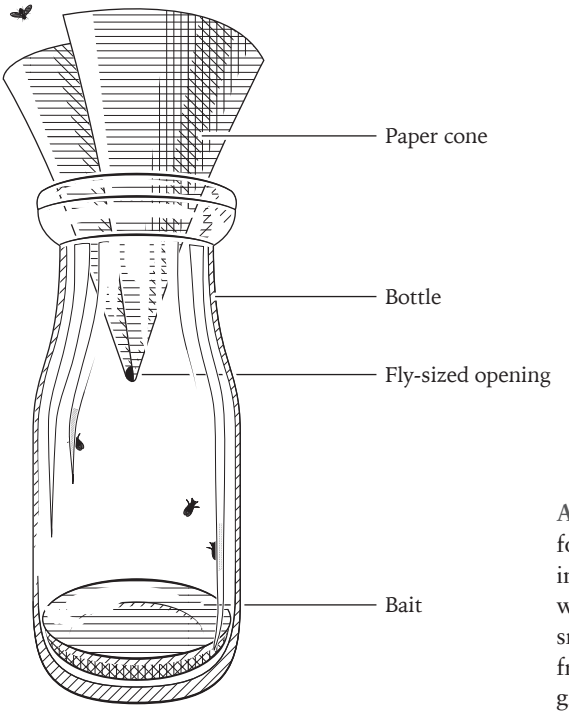
APPENDIX A

HOW TO MAKE A FLYTRAP

Although researchers take great care to contain fruit flies in a lab setting, some inevitably get free, and once free, the flies will follow enticing scents to places they are unwelcome, such as a lunchroom or a yeast research lab. *Drosophila* researchers are not likely to bring insecticides into the lab—we are for the most part trying very hard to keep fruit flies alive, after all, so that we can perform genetic crosses and do other types of studies. But even the most dedicated fly researchers recognize that rogue flies can be a nuisance. We have developed an effective strategy for capturing flies that have escaped from their culture vials: we place simple flytraps in strategic locations around the lab. The same approach can be used in a home to get rid of an unwanted infestation or to collect wild flies for study. In the 2014 BioSCAN project, volunteers in Los Angeles, California, placed flytraps in their backyards as part of a species survey. These backyard collections led to identification of several new species of phorid flies (Twilley 2015), as well as the observation that *Drosophila flavohirta,* a species not previously found in the Americas, has taken up residence in Los Angeles (Grimaldi et al. 2015).

To make a trap, first gather the following: an empty bottle, a rubber band or tape, and a tablespoon of something attractive to the flies, such as fruit juice, cider, champagne, wine, red wine vinegar, mushy banana, tired grapes, or overripe mango. The materials should be assembled as shown, then placed in areas frequented by the flies. The paper cone or a similar cover with a single small opening is essential: an open jar constitutes a fly feeder rather than a flytrap. The opening in the paper cone or cover should be just large enough for the small flies to fit through. Flies will be attracted by the bait and enter the chamber. Once inside, they are unlikely to try to escape, and even if they try, it will be next to impossible for them to find the tiny hole through which they entered.

This is a live trap method. To get rid of the flies, seal and throw away the trap within a few days. If forgotten, the trap is likely to become the



A flytrap. Good baits for catching *Drosophila* include apple juice, wine, or vinegar, or small bits of overripe fruits such as bananas, grapes, or mangos.

birthplace of a next generation of flies. Before tossing the trap out, the curious might examine the flies to determine what species of flies were caught and whether they have unusual or interesting attributes. Should you decide to culture the flies, sliced bananas with a sprinkle of baker's yeast should suffice as a food source, and a piece of cloth secured with a rubber band or a plug of cotton can be used to stopper the top without depriving the flies of oxygen. Be careful not to let the food dry out or expose the flies to prolonged heat or cold. To anesthetize the flies for close examination, place the trap on ice for five to ten minutes. If the food is very mushy, put the trap on its side, so the anesthetized flies do not get stuck in the food, or use a funnel to transfer the flies to an empty bottle before placing them on ice. A small paintbrush, makeup brush, or crab pick can be used as a pusher, and a magnifying glass, macro lens attachment on a camera, or smartphone-compatible microscope can help you inspect your flies closely. Further information, including food recipes and video demonstrations, can be found online. Who knows? Isolation of a spontaneous mutation, observation of an interesting behavior, or some other chance finding might launch a groundbreaking study.

APPENDIX B

COMPARABLE ORGANS IN HUMANS AND FLIES

Human cell type, tissue, or organ	Comparable cell type, tissue, or organ in adult *Drosophila*
Nervous and sensory systems	
Brain and central nervous system (CNS)	Brain and CNS
Peripheral nervous system (PNS)	PNS
Spinal cord	Ventral nerve cord
Neuromuscular junctions (NMJs), specialized connections between nerve and muscle cells	NMJs with a similar structure and components
Eyes (color vision)	Compound eyes (color vision) (Montell 2012), including a lens structure (Charlton-Perkins et al. 2011)
Ears (sense of hearing)	Regions of the antennae (Johnston's organ) (Albert and Gopfert 2015; Ishikawa and Kamikouchi 2015; Senthilan et al. 2012)
Nose (sense of smell)	Third antennal segment (funiculus) and maxillary palps (Martin et al. 2013; Stocker 1994)
Taste buds	Taste sensilla on the labellum of the proboscis, at the tips of the legs and wings, and in females, at the tip of the abdomen (Montell 2009; Stocker 1994); detailed map in (Freeman and Dahanukar 2015)
Skin (sense of touch)	Hairlike bristles (sensory bristles) (Lumpkin et al. 2010)
Circulation, blood, and lymph systems	
Aorta	Dorsal vessel (anterior portion) (Zikova et al. 2003)
Heart	Dorsal vessel (posterior portion) with cardial cells that rhythmically contract (Bodmer 1995; Zikova et al. 2003)
Vascular system and lungs (branched architecture, oxygen exchange role)	Tracheal system (branched architecture, oxygen exchange role) (Andrew and Ewald 2010; Roeder et al. 2012)
Lymph glands	Lymph glands (Evans et al. 2003; Gold and Bruckner 2014)
Self-renewing blood cell progenitors (hematopoietic progenitors), and derivative blood cell types	Self-renewing hemocytes and lymph resident hemocytes, and derivative blood cells types (Evans et al. 2003; Gold and Bruckner 2014; Meister and Lagueux 2003)

Digestion, detoxification, fat storage, and fat metabolism systems	
Salivary gland	Salivary gland
Stomach	Midgut (Lemaitre and Miguel-Aliaga 2013; Singh et al. 2011)
Large intestine, rectum, and anus	Hindgut, rectum, and anus (Apidianakis and Rahme 2011)
Intestinal mucosa	Peritrophic membrane (Lemaitre and Miguel-Aliaga 2013)
Kidneys	Malpighian tubules (Beyenbach, Skaer, and Dow 2010)
Kidney podocytes	Cardiac nephrocytes (Gee et al. 2015; Weavers et al. 2009)
Liver (detoxification role of)	Fat body and Malpighian tubules (detoxification roles of) (Yang et al. 2007)
Liver (fat storage role of) and white adipose tissue	Fat body (fat storage role of) (Arrese and Soulages 2010)
Liver (fat metabolism role of)	Oenocytes (Chatterjee et al. 2014; Gutierrez et al. 2007)
Hormonal and glandular systems	
Liver (IGF-1 production by)	Fat body (Dilp6 production by) (Okamoto et al. 2009; Slaidina et al. 2009)
Pancreas (insulin production and secretion by)	Insulin-producing cells (IPCs) of the brain (Rulifson et al. 2002)
Neuroendocrine system	Pars intercerebralis, pars lateralis, and connections thereof to the ring gland (Pfeiffer and Homberg 2014)
Endocrine glands	Ring gland (prothoracic gland, corpora allata and corpus cardiaca) (Pfeiffer and Homberg 2014)
Thyroid gland	Corpora allata (Flatt et al. 2006; Gade, Hoffmann, and Spring 1997)
Prostate gland	Accessory gland (Ito et al. 2014)

(*continued*)

Human cell type, tissue, or organ	Comparable cell type, tissue, or organ in adult *Drosophila*
Blood, skin, and immune protective systems	
Skin	Cuticle (Patterson et al. 2013)
Macrophages	Plasmatocytes (Gold and Bruckner 2014)
Muscle systems	
Skeletal muscles (e.g., of the legs, arms)	Muscles (e.g., of the legs, wings)
Smooth muscles (e.g., surrounding the digestive system)	Visceral muscles (e.g., surrounding the digestive system)
Limbs	
Arms and legs	Legs, halteres, and wings
Reproductive systems	
Ovaries	Ovaries
Testes	Testes

APPENDIX C

SELECTED GENETIC SCREENS PERFORMED IN *DROSOPHILA*

Many genetic screens have been undertaken by fly researchers over the years, and a variety of genetic tricks can be used in *Drosophila* as part of an overall screen strategy. D. St Johnston's "The art and design of genetic screens: *Drosophila melanogaster*" provides instructive examples of *Drosophila* genetic screens and screening methods (St Johnston 2002). Comprehensive overviews of genetic approaches include (Venken and Bellen 2012). The examples below represent a variety of screen strategies and assays, leading to results impacting our understanding of diverse biological topics.

MAKE A FLY

In their 1980 article "Mutations affecting segment number and polarity in Drosophila," E. Wieschaus and C. Nüsslein-Volhard reported an EMS mutagenesis screen in which they isolated lethal mutations that result in disruption of larval development, a first screen aimed at "saturating" or identifying all genes that can be mutated to generate the phenotype under study (Nüsslein-Volhard and Wieschaus 1980; Wieschaus and Nüsslein-Volhard 2016). In articles published in 1989 and 1991, T. Schüpbach and E. Wieschaus extended this analysis to identification of nonlethal mutations in genes required in mothers to produce offspring that develop normally ("maternal-effect" genes) (Schüpbach and Wieschaus 1989; 1991). As reported in 1989 and 1996, N. Perrimon and colleagues used the "dominant female sterile" technique to further extend the analysis by identifying essential genes required in mothers to produce offspring that develop normally (Perrimon, Engstrom, and Mahowald 1989; Perrimon et al. 1996). These screens are discussed in Chapter 3.

BEHAVE LIKE A FLY

In their 1971 article, "Clock mutants of *Drosophila melanogaster,*" R. J. Konopka and S. Benzer reported an EMS mutagenesis screen in which they isolated *period* mutant flies with altered day-night activity cycles, demonstrating that it is possible to screen for and isolate single-gene mutations that affect complex behaviors such as circadian rhythms (Konopka and Benzer 1971). In 1980, D. Suzuki and colleagues reported an EMS screen in which they isolated flies with defects in various other behaviors, including flight, activity levels, and stress sensitivity (Homyk, Szidonya, and Suzuki 1980). In 1989, C. Woodard, J. Carlson and colleagues used EMS and X-rays in a screen for mutations affecting olfaction, or the sense of smell (Woodard et al. 1989). Work by Benzer and others identified the molecular mechanisms underlying control of circadian rhythms and, more broadly, opened the door to a new way of thinking about how genes affect behavior (Chapter 8; [Benzer 1971]). Sequencing of the *period* gene (Zehring et al. 1984; Bargiello et al. 1984) and subsequent studies of genes like *period* that are required for the control of circadian rhythms also led to the awarding of a Nobel Prize in physiology or medicine to J. C. Hall, M. Rosbash, and M. W. Young (Nobel Media 2017).

GET DRUNK LIKE A FLY

In a 1998 report titled "Ethanol intoxication in *Drosophila* . . . ," U. Heberlein and colleagues reported a screen of P-element transposon insertion strains for flies that have altered sensitivity to inebriation by alcohol. To do the screen, they put flies into a sorting apparatus called an "inebriometer," in which the more drunk a fly gets, the more likely it is to fall to the bottom of the contraption (Moore et al. 1998). The mutation characterized in the first paper reporting results from the screen was named *cheapdate* at the time, but turned out to be a mutant allele of a previously identified gene, *amnesiac*. The results of this screen and other studies demonstrated that the fly can be used to study genetic components of alcohol and drug addiction (Chapter 8).

AMP UP GENE ACTIVITY

In a 1998 report titled "Systematic gain-of-function genetics in *Drosophila,*" P. Rørth and colleagues developed a P-element with the ability to

turn on or increase expression of a gene adjacent to the site of insertion, generated a collection of random insertions of the element (the "EP" collection), and screened for insertions that affect development of the eye (Rørth et al. 1998). Studies like this are complementary to more typical forward or reverse genetic screens, in which gene function is reduced or eliminated. For some genes, inducing or up-regulating activity tells us more or provides additional information as compared with observing what happens when the gene is turned off. The application of the CRISPR activation approach in *Drosophila* (Lin et al. 2015) opened a door to targeted and systematic activation-based screens.

LOOK AT WHAT'S EASY TO SEE

As an easily viewed, well-characterized, external structure that is not required for viability or fertility of the fly, the eye serves as a convenient tissue in which to screen for mutant phenotypes. In 1991, M. A. Simon, G. M. Rubin, and colleagues reported a dominant modifier screen for mutations that worsen the phenotype of a mutation in the *sevenless* gene that affects the texture of the eye (Chapter 3; [Simon et al. 1991]). The study identified a fly ortholog of the human cancer-related gene *RAS* as a participant in transduction of a signal received by the Sevenless receptor. In 2001, I. K. Hariharan and colleagues reported the results of a screen using a genetic trick called "FRT-FLP" (Golic and Lindquist 1989) to generate flies in which only eye tissue is mutant (Moberg et al. 2001). Similar screens by three groups contributed to the identification of genes required for control of organ size (Chapter 4; [Pagliarini and Xu 2003; Xu et al. 1995; Tapon et al. 2001]). In 2006, U. Banarjee and colleagues used the same approach to make flies in which only the eye was mutant, this time looking for mutations affecting the mitochondria, the site of energy production of a cell (Liao et al. 2006).

LOOK AT WHAT'S LIVING

The fact that a homozygous mutant fly survives to adulthood does not mean it is "normal" in all senses. Sets of flies that are known to carry mutations but are viable can provide a starting test set for screens of adult mutant phenotypes, such as differences in behavior. In 2003, W. D. Tracey and colleagues used the set of homozygous viable insertions in the Rørth EP collection (Rørth et al. 1998) to identify flies with altered responses to

pain (Tracey et al. 2003). In 2006, K. M. Beckingham and colleagues screened another collection of homozygous viable mutant flies, the Zuker collection (Koundakjian et al. 2004), and identified flies with altered responses to gravity (Armstrong et al. 2006). The Zuker collection was also screened for mutations affecting sleep by A. Sehgal and colleagues (Wu et al. 2008).

EXPLOIT CULTURED CELLS

Drosophila "cultured cells" are cells that have been dissociated or removed from a fly (such as a fly embryo), then found to be capable of growing and dividing, more or less indefinitely, if kept alive in liquid food (culture media) designed to mimic conditions within the body (Echalier 1997). In 2003, A. Kiger, N. Perrimon, and colleagues reported the first genome-wide screen using RNAi to reduce RNA levels in *Drosophila* cultured cells. The researchers used the technique to find genes required for cell shape (Kiger et al. 2003). In 2006, several groups reported using the same approach in *Drosophila* cell studies that led to identification in the human genome of long-sought but previously undiscovered genes encoding or controlling calcium channel activity (Feske et al. 2006; Vig et al. 2006; Zhang et al. 2006). The approach has also been effective in identifying new components of the Hh, Wnt, JAK/STAT, and other signal transduction pathways, as in studies led by K. Basler, P. A. Beachy, M. Boutros, N. Perrimon, M. P. Zeidler, and others (Baeg, Zhou, and Perrimon 2005; DasGupta et al. 2005; Muller et al. 2005; Nybakken et al. 2005; Lum et al. 2003; Vidal et al. 2010).

MAKE THEM GLOW

Genes that encode fluorescent proteins can be expressed in specific organs or cell types, and the fluorescent proteins observed with ultraviolet light. This facilitates screening for changes in the number or location of cells that express the fluorescent protein. In 2003, R. A. Pagliarini and T. Xu made flies in which only the eye tissue was mutant, and in which that tissue was marked with a green fluorescent protein, then asked whether any of the mutations gave the cells the ability to invade other tissues, similar to metastasis in cancer, by searching for the fluorescent signal in other parts of the fly (Pagliarini and Xu 2003). Large-scale screens by four groups used fluorescent proteins to uncover new genes required to make a heart. All four studies used expression in heart precursors of a green fluorescent protein as a marker of heart progenitor cells; one additionally included a

red-glowing fluorescent protein marker (Tao, Christiansen, and Schulz 2007; Yi et al. 2006; Hollfelder, Frasch, and Reim 2014; Drechsler et al. 2013). Several of the genes identified in these screens are required for formation or stability of the cardiac extracellular matrix, as discussed in (Frasch 2016).

MAKE THEM FAT

In part as a response to rising interest in human obesity and diabetes (Chapter 4), fly researchers are exploring the contribution of genes to changes related to accumulation of fat, changes in blood sugar levels, and other relevant phenotypes. In 2010, J. M. Penninger and colleagues reported a genome-wide screen of *Drosophila* in which they used RNAi to reduce RNA levels and identified genes involved in "adiposity" or fat deposits (Pospisilik et al. 2010). The screen results and follow-up tests implicated the Hh pathway in formation of fly and mammalian fat cells, and provide a long list of candidate genes that might be relevant to obesity in humans. The screen is one among many that exemplify an emphasis on using the fly as a model to study physiology and metabolism, in particular as they relate to human disease, that has emerged in the post-genomic era.

FOCUS ON STEM CELLS

The fly is an excellent system in which to explore the functions of stem cells, which fill in for losses in order to make repairs, maintain health, and reproduce (Chapter 10). In 2011, J. A. Knoblich and colleagues reported a screen looking at neuroblasts, a type of stem cell associated with the nervous system, by expressing RNAi reagents only in that cell type (Neumüller et al. 2011). In 2014, N. Perrimon and colleagues reported an RNAi screen exploring germline stem cells in the ovary (Yan et al. 2014) and in 2016, a similar screen of germline stem cells in the testis was reported by C. Tong and colleagues (Yu et al. 2016). Comparison of the results of these screens can help to define which genes are commonly required for stem cell functions and which are required in specific cell types.

LIMIT THE POOL, THEN GO FISHING

A genome-wide screen is not always practical. There are many ways to limit the initial test set to cut down on the work without unduly limiting

the chance for discovery. Screens that began with a set of mutant but viable flies provide an example of one strategy to limiting the number of flies screened. Another approach is exemplified by a 2016 report by S. Bhat and W. D. Jones. These researchers designed a "two-tiered" screen in which they first expressed specific micro RNAs (miRNAs), small RNA regulators of protein-coding genes, and observed the effects of that expression; next used the results to define a set of genes that might be targeted by the miRNAs; and then used RNAi to ask whether these target genes are relevant to expression of odorant receptors (smell receptors) in sensory neurons (Bhat and Jones 2016).

GET NERVOUS

In a 2014 study, H. J. Bellen and colleagues used EMS as a mutagen to identify recessive mutations on the X chromosome that result in lethality, then screened the collection for mutations that affect the nervous system and identified 165 genes that met their criteria (Yamamoto et al. 2014). Many of the genes have orthologs in humans that are associated with disease. The team leveraged the results they obtained in flies to provide new information regarding what had been puzzling cases of human inherited disorders.

As these examples show, the types of approaches that can be used and the mutant phenotypes that can be detected, even in large-scale, genome-wide screens, are wonderfully diverse. New innovations, including in the areas of genetic perturbation, lab automation, image or video capture and analysis, and labeling and detection of fluorescent probes, are facilitating a next generation of genetic screens in *Drosophila*. Next steps following any genetic screen include identifying how many and what genes are responsible for the phenotypes observed; re-testing genes identified in the primary screen using additional assays; comparing new results with what is known already for *Drosophila* and for orthologs in other species; and figuring out what if any novel findings have emerged and what their broader implications might be. As these inevitably lead to more questions, the eventual next step might be to perform another screen, such as to identify modifiers of a mutation isolated in the initial study. If geneticists had a motto it might be this: screen, validate, integrate (with previous knowledge), and repeat.

RECOMMENDED READING

Endless Forms Most Beautiful, Sean B. Carroll

Essays Upon Heredity, August Weismann

FlyBook, an article series in the journal *Genetics*

Heredity and Variation, L. C. Dunn

A History of Genetics, A. H. Sturtevant

The Life of the Fly, Jean-Henri Fabre

Lords of the Fly, Robert E. Kohler

The Making of a Fly, Peter A. Lawrence

The Natural History of Flies, Harold Oldroyd

Time, Love, Memory, Jonathan Weiner

ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
Ago3	*Argonaute 3* (gene)
Ago3	Argonaute 3 (protein)
ALS	amyotrophic lateral sclerosis
AMPs	antimicrobial peptides
ANT-C	*Antp* Complex
Antp	*Antennapedia* (gene)
Antp	Antennapedia (protein)
ATP	adenosine triphosphate
bor	*belphegor*
BX-C	*Bithorax* Complex
C. elegans	*Caenorhabditis elegans*
CFTR	*Cystic fibrosis transmembrane conductance regulator* (human gene)
ci	*cubitus interruptus* (gene)
Ci	Cubitus interruptus (protein)
cM	centiMorgan
CMT2B	Charcot-Marie-Tooth disease type 2B
CRISPR	clustered regularly interspaced short palindromic repeats
dach	*dachshund* (gene)
Dach	Dachshund (protein)
DDT	dichlorodiphenyltrichloroethane
dgo	*diego* (gene)
Dgo	Diego (protein)
DHH	*desert hedgehog* (human gene)
Dilps	*Drosophila* insulin-like peptides
DIS	*Drosophila* Information Service
DNA	deoxyribonucleic acid
ds	*dachsous* (gene)

Ds	Dachsous (protein)
dsh	*disheveled* (gene)
Dsh	Disheveled (protein)
EMS	ethyl methanesulfonate
ENCODE	encyclopedia of DNA elements
ERG	electroretinogram
esc	*extra sex combs*
evo-devo	evolutionary developmental biology
ey	*eyeless* (gene)
Ey	Eyeless (protein)
eya	*eyes absent* (gene)
Eya	Eyes absent (protein)
EYA	human Ey/Eya-related transcriptional pathway
F_1	first filial generation (offspring of the P_0)
F_2	second filial generation (grandchildren of the P_0)
FDA	U.S. Food and Drug Administration
fj	*four-jointed* (gene)
Fj	Four-jointed (protein)
FLP	yeast flippase protein
FlyMAD	Fly Mind Altering Device
fmi/stan	*flamingo/starry night* (gene)
Fmi	Flamingo/Starry night (protein)
FRT	flippase recognition target
FTP	file transfer protocol
Fw	Furrowed (protein)
Fy	Fuzzy (protein)
fz	*frizzled* (gene)
Fz	Frizzled (protein)
FZDs	Frizzled-related receptors
GSCs	germline stem cells
hh	*hedgehog* (gene)
Hh	Hedgehog (protein)
Hh pathway	Hh signal transduction pathway
Hox	Homeobox domain-containing
HTTP	hypertext transfer protocol
IHH	*Indian hedgehog* (human gene)
In	Inturned (protein)
IPCs	insulin-producing cells

iPSCs	induced pluripotent stem cells
JO	Johnston's organ
K^+	potassium ion
m	*miniature* (gene)
miRNAs	micro RNAs
mm	millimeters
Msc	*Multiple sex comb* (mutation in *Scr*)
mwh	*multiple wing hairs* (gene)
Mwh	Multiple wing hairs (protein)
NIH	U.S. National Institutes of Health
P_0	parental group (parents of the F_1)
PAX	paired box domain-containing
Pc	*Polycomb*
PCP	planar cell polarity
pk	*prickle* (gene)
Pk	Prickle (protein)
PRC	Polycomb repressive complex
ptc	*patched* (gene)
Ptc	Patched (protein)
r	*rudimentary* (gene)
RNA	ribonucleic acid
RNAi	RNA interference
ROK	Rho-associated kinase (protein)
Scr	*Sex combs reduced*
Scx	*Sex combs extra*
SHH	*Sonic hedgehog* (human gene)
SMART	somatic mutation and recombination test
smo	*smoothened*
Smo	Smoothened protein
SMO	human Smoothened protein
so	*sine oculis* (gene)
So	Sine oculis (protein)
Sos	*Son of sevenless* (gene)
Sos	Son of sevenless (protein)
TBI	traumatic brain injury
TLR	Toll-like receptor
toy	*twin of eyeless* (gene)
Toy	Twin of eyeless (protein)

trp	*transient receptor potential* (gene)
TRP	Transient Receptor Potential (protein)
TRP family	TRP-related gene or protein family
TrxG	Trithorax Group
TSS	transcription start site
UAS	upstream activation sequence
UDN	NIH Undiagnosed Diseases Network
v	*vermilion* (gene)
Vang	*Van Gogh* (gene)
Vang	Van Gogh (protein)
w	*white* (gene)
Wnt	*Drosophila wingless* and mouse *int1*-related
y	*yellow* (gene)

REFERENCES

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, S. E. Scherer, et al. 2000. "The genome sequence of *Drosophila melanogaster*." *Science* 287 (5461): 2185–95. doi: 10.1126/science.287.5461.2185.

Alaraby, M., B. Annangi, A. Hernandez, A. Creus, and R. Marcos. 2015. "A comprehensive study of the harmful effects of ZnO nanoparticles using *Drosophila melanogaster* as an in vivo model." *J Hazard Mater* 296:166–74. doi: 10.1016/j.jhazmat.2015.04.053.

Alaraby, M., E. Demir, A. Hernandez, and R. Marcos. 2015. "Assessing potential harmful effects of CdSe quantum dots by using *Drosophila melanogaster* as in vivo model." *Sci Total Environ* 530–31:66–75. doi: 10.1016/j.scitotenv.2015.05.069.

Alberi, L., S. E. Hoey, E. Brai, A. L. Scotti, and S. Marathe. 2013. "Notch signaling in the brain: in good and bad times." *Ageing Res Rev* 12 (3): 801–14. doi: 10.1016/j.arr.2013.03.004.

Albert, J. T., and M. C. Gopfert. 2015. "Hearing in *Drosophila*." *Curr Opin Neurobiol* 34:79–85. doi: 10.1016/j.conb.2015.02.001.

Alderson, T. 1965. "Chemically induced delayed germinal mutation in *Drosophila*." *Nature* 207 (993): 164–67. doi: 10.1038/207164a0.

Alpatov, W. W., and Raymond Pearl. 1929. "Experimental studies on the duration of life. XII. Influence of temperature during the larval period and adult life on the duration of the life of the imago of *Drosophila melanogaster*." *American Naturalist* 63 (684): 37–67. doi: 10.1086/280236.

Amoyel, M., and E. A. Bach. 2014. "Cell competition: how to eliminate your neighbours." *Development* 141 (5): 988–1000. doi: 10.1242/dev.079129.

Anaka, M., C. D. MacDonald, E. Barkova, K. Simon, R. Rostom, R. A. Godoy, A. J. Haigh, I. A. Meinertzhagen, and V. Lloyd. 2008. "The *white* gene of *Drosophila melanogaster* encodes a protein with a role in courtship behavior." *J Neurogenet* 22 (4): 243–76. doi: 10.1080/01677060802309629.

Anderson, D. J., and R. Adolphs. 2014. "A framework for studying emotions across species." *Cell* 157 (1): 187–200. doi: 10.1016/j.cell.2014.03.003.

Anderson, K. V., G. Jürgens, and C. Nüsslein-Volhard. 1985. "Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role

of the *Toll* gene product." *Cell* 42 (3): 779–89. doi: 10.1016/0092-8674(85)90274-0.

Andrew, D. J., and A. J. Ewald. 2010. "Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration." *Dev Biol* 341 (1): 34–55. doi: 10.1016/j.ydbio.2009.09.024.

Angus, J. 1974. "Genetic control of activity, preening, and the response to a shadow stimulus in *Drosophila melanogaster*." *Behav Genet* 4 (4): 317–29. doi: 10.1007/BF01066153.

Anholt, R. R., and T. F. Mackay. 2012. "Genetics of aggression." *Annu Rev Genet* 46:145–64. doi: 10.1146/annurev-genet-110711-155514.

Apidianakis, Y., and L. G. Rahme. 2011. "*Drosophila melanogaster* as a model for human intestinal infection and pathology." *Dis Model Mech* 4 (1): 21–30. doi: 10.1242/dmm.003970.

Arctic Desert and Tropic Information Center. 1944. The mosquito and fly problem in the Arctic. U.S. Army Air Forces, Arctic. New York: Arctic, Desert and Tropic Information Center as reproduced by the Training Aids Division, Ofice of the Assistant Chief of Air Staff, Training Headquarters Army Air Forces.

Armstrong, J. D., M. J. Texada, R. Munjaal, D. A. Baker, and K. M. Beckingham. 2006. "Gravitaxis in *Drosophila melanogaster*: a forward genetic screen." *Genes Brain Behav* 5 (3): 222–39. doi: 10.1111/j.1601-183X.2005.00154.x.

Arrese, E. L., and J. L. Soulages. 2010. "Insect fat body: energy, metabolism, and regulation." *Annu Rev Entomol* 55:207–25. doi: 10.1146/annurev-ento-112408-085356.

Ashburner, M., and R. Drysdale. 1994. "FlyBase—the *Drosophila* genetic database." *Development* 120 (7): 2077–79.

Aso, Y., D. Hattori, Y. Yu, R. M. Johnston, N. A. Iyer, T. T. Ngo, H. Dionne, L. F. Abbott, R. Axel, H. Tanimoto, and G. M. Rubin. 2014. "The neuronal architecture of the mushroom body provides a logic for associative learning." *Elife* 3:e04577. doi: 10.7554/eLife.04577.

Atwood, S. X., R. J. Whitson, and A. E. Oro. 2014. "Advanced treatment for basal cell carcinomas." *Cold Spring Harb Perspect Med* 4 (7): a013581. doi: 10.1101/cshperspect.a013581.

Auerbach, C., J. M. Robson, and J. G. Carr. 1947. "The chemical production of mutations." *Science* 105 (2723): 243–47. doi: 10.1126/science.105.2723.243.

Aughey, G. N., and J. L. Liu. 2015. "Metabolic regulation via enzyme filamentation." *Crit Rev Biochem Mol Biol* 51 (4): 282–93. doi: 10.3109/10409238.2016.1172555.

Auluck, P. K., H. Y. Chan, J. Q. Trojanowski, V. M. Lee, and N. M. Bonini. 2002. "Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease." *Science* 295 (5556): 865–68. doi: 10.1126/science.1067389.

Azuma, N., Y. Yamaguchi, H. Handa, K. Tadokoro, A. Asaka, E. Kawase, and M. Yamada. 2003. "Mutations of the PAX6 gene detected in patients with a

variety of optic-nerve malformations." *Am J Hum Genet* 72 (6): 1565–70. doi: 10.1086/375555.

Baeg, G. H., R. Zhou, and N. Perrimon. 2005. "Genome-wide RNAi analysis of JAK/STAT signaling components in *Drosophila*." *Genes Dev* 19 (16): 1861–70. doi: 10.1101/gad.1320705.

Baena-Lopez, L. A., H. Nojima, and J. P. Vincent. 2012. "Integration of morphogen signalling within the growth regulatory network." *Curr Opin Cell Biol* 24 (2): 166–72. doi: 10.1016/j.ceb.2011.12.010.

Baets, J., P. De Jonghe, and V. Timmerman. 2014. "Recent advances in Charcot-Marie-Tooth disease." *Curr Opin Neurol* 27 (5): 532–40. doi: 10.1097/WCO.0000000000000131.

Baillon, L., and K. Basler. 2014. "Reflections on cell competition." *Semin Cell Dev Biol* 32:137–44. doi: 10.1016/j.semcdb.2014.04.034.

Balbiani, E. G. 1881. "Sur la structure du noyau des cellules salivaires chez les larves de *Chironomus*." *Zoologische Anzeiger* 4:637–641.

Bargiello TA, Jackson FR, Young MW. 1984. "Restoration of circadian behavioural rhythms by gene transfer in *Drosophila*." *Nature* 312 (5996): 752–54. doi: 10.1038/312752a0.

Barrows, W. M. 1907. "The reactions of the Pomace fly, *Drosophila ampelophila* loew, to odorous substances." *Journal of Experimental Zoology* 4 (4): 515–37. doi: 10.1002/jez.1400040403.

Barzel, B., and A. L. Barabasi. 2013. "Universality in network dynamics." *Nat Phys* 9. doi: 10.1038/nphys2741.

Bateson, W. 1909. *Mendel's Principles of Heredity.* Cambridge: At the University Press.

Bath, D. E., J. R. Stowers, D. Hormann, A. Poehlmann, B. J. Dickson, and A. D. Straw. 2014. "FlyMAD: rapid thermogenetic control of neuronal activity in freely walking *Drosophila*." *Nat Methods* 11 (7): 756–62. doi: 10.1038/nmeth.2973.

Beal, M. F. 2001. "Experimental models of Parkinson's disease." *Nat Rev Neurosci* 2 (5): 325–34. doi: 10.1038/35072550.

Beckage, N. E. 2007. *Insect Immunology.* Vol. 1. New York: Academic Press.

Beira, J. V., and R. Paro. 2016. "The legacy of *Drosophila* imaginal discs." *Chromosoma* 125 (4): 573–92. doi: 10.1007/s00412-016-0595-4.

Bellaiche, Y., I. The, and N. Perrimon. 1998. "Tout-velu is a *Drosophila* homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion." *Nature* 394 (6688): 85–88. doi: 10.1038/27932.

Bellen, H. J., and S. Yamamoto. 2015. "Morgan's legacy: fruit flies and the functional annotation of conserved genes." *Cell* 163 (1): 12–14. doi: 10.1016/j.cell.2015.09.009.

Benzer, S. 1971. "From the gene to behavior." *JAMA* 218 (7): 1015–22. doi: 10.1001/jama.1971.03190200047010.

Berman, G. J., D. M. Choi, W. Bialek, and J. W. Shaevitz. 2014. "Mapping the stereotyped behaviour of freely moving fruit flies." *J R Soc Interface* 11 (99). doi: 10.1098/rsif.2014.0672.

Berry-Kravis, E., D. Hessl, S. Coffey, C. Hervey, A. Schneider, J. Yuhas, J. Hutchison, et al. 2009. "A pilot open label, single dose trial of fenobam in adults with fragile X syndrome." *J Med Genet* 46 (4): 266–71. doi: 10.1136/jmg.2008.063701.

Berry-Kravis, E., A. Sumis, C. Hervey, M. Nelson, S. W. Porges, N. Weng, I. J. Weiler, and W. T. Greenough. 2008. "Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome." *J Dev Behav Pediatr* 29 (4): 293–302. doi: 10.1097/DBP.0b013e31817dc447.

Beyenbach, K. W., H. Skaer, and J. A. Dow. 2010. "The developmental, molecular, and transport biology of Malpighian tubules." *Annu Rev Entomol* 55:351–74. doi: 10.1146/annurev-ento-112408-085512.

Bharucha, K. N. 2009. "The epicurean fly: using *Drosophila melanogaster* to study metabolism." *Pediatr Res* 65 (2): 132–37. doi: 10.1203/PDR.0b013e318191fc68.

Bhat, S., and W. D. Jones. 2016. "An accelerated miRNA-based screen implicates Atf-3 in *Drosophila* odorant receptor expression." *Sci Rep* 6:20109. doi: 10.1038/srep20109.

Bian, G., Y. Xu, P. Lu, Y. Xie, and Z. Xi. 2010. "The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*." *PLoS Pathog* 6 (4): e1000833. doi: 10.1371/journal.ppat.1000833.

Bodmer, R. 1995. "Heart development in *Drosophila* and its relationship to vertebrates." *Trends Cardiovasc Med* 5 (1): 21–28. doi: 10.1016/1050-1738(94)00032-Q.

Boekhoff-Falk, G. 2005. "Hearing in *Drosophila*: development of Johnston's organ and emerging parallels to vertebrate ear development." *Dev Dyn* 232 (3): 550–58. doi: 10.1002/dvdy.20207.

Bohni, R., J. Riesgo-Escovar, S. Oldham, W. Brogiolo, H. Stocker, B. F. Andruss, K. Beckingham, and E. Hafen. 1999. "Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4." *Cell* 97 (7): 865–75. doi: 10.1016/S0092-8674(00)80799-0.

Bonilla-Ramirez, L., M. Jimenez-Del-Rio, and C. Velez-Pardo. 2011. "Acute and chronic metal exposure impairs locomotion activity in *Drosophila melanogaster*: a model to study Parkinsonism." *Biometals* 24 (6): 1045–57. doi: 10.1007/s10534-011-9463-0.

Bonvini, S. J., M. A. Birrell, J. A. Smith, and M. G. Belvisi. 2015. "Targeting TRP channels for chronic cough: from bench to bedside." *Naunyn Schmiedebergs Arch Pharmacol* 388 (4): 401–20. doi: 10.1007/s00210-014-1082-1.

Boulan, L., M. Milan, and P. Leopold. 2015. "The systemic control of growth." *Cold Spring Harb Perspect Biol* 7 (12). doi: 10.1101/cshperspect.a019117.

Boulanger, N., L. Ehret-Sabatier, R. Brun, D. Zachary, P. Bulet, and J. L. Imler. 2001. "Immune response of *Drosophila melanogaster* to infection with the flagellate

parasite *Crithidia* spp." *Insect Biochem Mol Biol* 31 (2): 129–37. doi: 10.1016/S0965-1748(00)00096-5.

Brand, A. H., and N. Perrimon. 1993. "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes." *Development* 118 (2): 401–15. http://dev.biologists.org/content/118/2/401.

Bridges, C. B. 1914. "Direct proof through non-disjunction that the sex-linked genes of drosophila are borne by the X-chromosome." *Science* 40 (1020): 107–9. doi: 10.1126/science.40.1020.107.

Bridges, C. B. 1935. "Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*." *Journal of Heredity* 26 (2): 60–64. doi: 10.1093/oxfordjournals.jhered.a104022.

Bridges, C. B., and K. S. Brehme. 1944. *The mutants of Drosophila melanogaster.* Carnegie Institution of Washington publication. Washington, DC: Carnegie Institution of Washington.

Bridges, C. B., and T. H. Morgan. 1923. *The third-chromosome group of mutant characters of Drosophila melanogaster.* Carnegie Institution of Washington publication. Washington, DC: Carnegie Institution of Washington.

Brinster, R. L., and J. W. Zimmermann. 1994. "Spermatogenesis following male germ-cell transplantation." *Proc Natl Acad Sci U S A* 91 (24): 11298–302. http://www.pnas.org/content/91/24/11298.

Brookheart, R. T., and J. G. Duncan. 2016a. "*Drosophila melanogaster*: an emerging model of transgenerational effects of maternal obesity." *Mol Cell Endocrinol* 435:20–28. doi: 10.1016/j.mce.2015.12.003.

Brookheart, R. T., and J. G. Duncan. 2016b. "Modeling dietary influences on offspring metabolic programming in *Drosophila melanogaster*." *Reproduction* 152 (3): R79–90. doi: 10.1530/REP-15-0595.

Brown, G. E., A. L. Mitchell, A. M. Peercy, and C. L. Robertson. 1996. "Learned helplessness in *Drosophila melanogaster*?" *Psychol Rep* 78 (3 Pt 1): 962. doi: 10.2466/pro.1996.78.3.962.

Brown, J. B., and S. E. Celniker. 2015. "Lessons from modENCODE." *Annu Rev Genomics Hum Genet* 16:31–53. doi: 10.1146/annurev-genom-090413-025448.

Brush, S. G. 1978. "Nettie M. Stevens and the discovery of sex determination by chromosomes." *Isis* 69 (247): 163–72. doi: 10.1086/352001.

Bryant, C. E., S. Orr, B. Ferguson, M. F. Symmons, J. P. Boyle, and T. P. Monie. 2015. "International Union of Basic and Clinical Pharmacology. XCVI. Pattern recognition receptors in health and disease." *Pharmacol Rev* 67 (2): 462–504. https://doi.org/10.1124/pr.114.009928.

Bryant, P. J. 1971. "Regeneration and duplication following operations in situ on the imaginal discs of *Drosophila melanogaster*." *Dev Biol* 26 (4): 637–51. https://doi.org/10.1016/0012-1606(71)90146-1.

Bryant, P. J., and P. Simpson. 1984. "Intrinsic and extrinsic control of growth in developing organs." *Quarterly Review of Biology* 59 (4): 387–415. http://www.jstor.org/stable/2828261.

Burglin, T. R., and M. Affolter. 2016. "Homeodomain proteins: an update." *Chromosoma* 125 (3): 497–521. doi: 10.1007/s00412-015-0543-8.

Burke, C., K. Trinh, V. Nadar, and S. Sanyal. 2017. "AxGxE: using flies to interrogate the complex etiology of neurodegenerative disease." *Curr Top Dev Biol* 121:225–51. doi: 10.1016/bs.ctdb.2016.07.007.

Bush, A. I. 2013. "The metal theory of Alzheimer's disease." *J Alzheimers Dis* 33 Suppl 1:S277–81. doi: 10.3233/JAD-2012-129011.

C. elegans Sequencing Consortium. 1998. "Genome sequence of the nematode *C. elegans*: a platform for investigating biology." *Science* 282 (5396): 2012–18.

Calendar, R. 1970. "The regulation of phage development." *Annu Rev Microbiol* 24:241–96. doi: 10.1146/annurev.mi.24.100170.001325.

Cardon, L. R., and T. Harris. 2016. "Precision medicine, genomics and drug discovery." *Hum Mol Genet*. doi: 10.1093/hmg/ddw246.

Carmona, E. R., C. Inostroza-Blancheteau, V. Obando, L. Rubio, and R. Marcos. 2015. "Genotoxicity of copper oxide nanoparticles in *Drosophila melanogaster*." *Mutat Res Genet Toxicol Environ Mutagen* 791:1–11. doi: 10.1016/j.mrgentox.2015.07.006.

Carpenter, D., D. M. Stone, J. Brush, A. Ryan, M. Armanini, G. Frantz, A. Rosenthal, and F. J. de Sauvage. 1998. "Characterization of two patched receptors for the vertebrate hedgehog protein family." *Proc Natl Acad Sci U S A* 95 (23): 13630–34. doi: 10.1073/pnas.95.23.13630.

Carpenter, G. H. 1928. *The biology of insects*. Series of Biological Handbooks. New York: Macmillan.

Carroll, S. B. 2005. *Endless forms most beautiful: the new science of evo devo and the making of the animal kingdom*. New York: Norton.

Carroll, T. J., and J. Yu. 2012. "The kidney and planar cell polarity." *Curr Top Dev Biol* 101:185–212. doi: 10.1016/B978-0-12-394592-1.00011-9.

Casal, J., P. A. Lawrence, and G. Struhl. 2006. "Two separate molecular systems, Dachsous/Fat and Starry night/Frizzled, act independently to confer planar cell polarity." *Development* 133 (22): 4561–72. doi: 10.1242/dev.02641.

Casas-Tinto, S., M. Torres, and E. Moreno. 2011. "The flower code and cancer development." *Clin Transl Oncol* 13 (1): 5–9. doi: 10.1007/s12094-011-0610-4.

Chae, J., M. J. Kim, J. H. Goo, S. Collier, D. Gubb, J. Charlton, P. N. Adler, and W. J. Park. 1999. "The *Drosophila* tissue polarity gene *starry night* encodes a member of the protocadherin family." *Development* 126 (23): 5421–29. http://dev.biologists.org/content/126/23/5421.

Chakravarti, L., E. H. Moscato, and M. S. Kayser. 2017. "Unraveling the neurobiology of sleep and sleep disorders using *Drosophila*." *Curr Top Dev Biol* 121:253–285. doi: 10.1016/bs.ctdb.2016.07.010.

Chao, H. T., M. Davids, E. Burke, J. G. Pappas, J. A. Rosenfeld, A. J. McCarty, T. Davis, L. et al. 2017. "A syndromic neurodevelopmental disorder caused by

de novo variants in EBF3." *Am J Hum Genet* 100 (1): 128–137. doi: 10.1016/j.ajhg.2016.11.018.

Charlton-Perkins, M., N. L. Brown, and T. A. Cook. 2011. "The lens in focus: a comparison of lens development in *Drosophila* and vertebrates." *Mol Genet Genomics* 286 (3–4): 189–213. doi: 10.1007/s00438-011-0643-y.

Chatterjee, D., S. D. Katewa, Y. Qi, S. A. Jackson, P. Kapahi, and H. Jasper. 2014. "Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in *Drosophila* oenocytes." *Proc Natl Acad Sci U S A* 111 (50): 17959–64. doi: 10.1073/pnas.1409241111.

Cherry, S., E. J. Jin, M. N. Ozel, Z. Lu, E. Agi, D. Wang, W. H. Jung, et al. 2013. "Charcot-Marie-Tooth 2B mutations in *rab7* cause dosage-dependent neurodegeneration due to partial loss of function." *Elife* 2:e01064. doi: 10.7554/eLife.01064.

Chi, T., M. S. Kim, S. Lang, N. Bose, A. Kahn, L. Flechner, S. D. Blaschko, et al. 2015. "A *Drosophila* model identifies a critical role for zinc in mineralization for kidney stone disease." *PLoS One* 10 (5): e0124150. doi: 10.1371/journal.pone.0124150.

Chiang, A. S., C. Y. Lin, C. C. Chuang, H. M. Chang, C. H. Hsieh, C. W. Yeh, C. T. Shih, et al. 2011. "Three-dimensional reconstruction of brain-wide wiring networks in *Drosophila* at single-cell resolution." *Curr Biol* 21 (1): 1–11. doi: 10.1016/j.cub.2010.11.056.

Chifiriuc, M. C., A. C. Ratiu, M. Popa, and A. A. Ecovoiu. 2016. "Drosophotoxicology: an emerging research area for assessing nanoparticles interaction with living organisms." *Int J Mol Sci* 17 (2): 36. doi: 10.3390/ijms17020036.

Chintapalli, V. R., J. Wang, and J. A. Dow. 2007. "Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease." *Nat Genet* 39 (6): 715–20. doi: 10.1038/ng2049.

Chouhan, A. K., C. Guo, Y. C. Hsieh, H. Ye, M. Senturk, Z. Zuo, Y. Li, et al. 2016. "Uncoupling neuronal death and dysfunction in *Drosophila* models of neurodegenerative disease." *Acta Neuropathol Commun* 4 (1): 62. doi: 10.1186/s40478-016-0333-4.

Chouinard, S. W., G. F. Wilson, A. K. Schlimgen, and B. Ganetzky. 1995. "A potassium channel beta subunit related to the aldo-keto reductase superfamily is encoded by the *Drosophila* Hyperkinetic locus." *Proc Natl Acad Sci U S A* 92 (15): 6763–67. http://www.pnas.org/content/92/15/6763.

Christiaens, J. F., L. M. Franco, T. L. Cools, L. De Meester, J. Michiels, T. Wenseleers, B. A. Hassan, E. Yaksi, and K. J. Verstrepen. 2014. "The fungal aroma gene ATF1 promotes dispersal of yeast cells through insect vectors." *Cell Rep* 9 (2): 425–32. doi: 10.1016/j.celrep.2014.09.009.

Christie, K. W., and D. F. Eberl. 2014. "Noise-induced hearing loss: new animal models." *Curr Opin Otolaryngol Head Neck Surg* 22 (5): 374–83. doi: 10.1097/MOO.0000000000000086.

Christie, K. W., E. Sivan-Loukianova, W. C. Smith, B. T. Aldrich, M. A. Schon, M. Roy, B. C. Lear, and D. F. Eberl. 2013. "Physiological, anatomical, and behavioral changes after acoustic trauma in *Drosophila melanogaster*." *Proc Natl Acad Sci U S A* 110 (38): 15449–54. doi: 10.1073/pnas.1307294110.

Ciabrelli, F., F. Comoglio, S. Fellous, B. Bonev, M. Ninova, Q. Szabo, A. Xuereb, et al. 2017. "Stable Polycomb-dependent transgenerational inheritance of chromatin states in *Drosophila*." *Nat Genet* 49 (6): 876–86. doi: 10.1038/ng.3848.

Clark, I. E., M. W. Dodson, C. Jiang, J. H. Cao, J. R. Huh, J. H. Seol, S. J. Yoo, B. A. Hay, and M. Guo. 2006. "*Drosophila pink1* is required for mitochondrial function and interacts genetically with *parkin*." *Nature* 441 (7097): 1162–66. doi: 10.1038/nature04779.

Claveria, C., G. Giovinazzo, R. Sierra, and M. Torres. 2013. "Myc-driven endogenous cell competition in the early mammalian embryo." *Nature* 500 (7460): 39–44. doi: 10.1038/nature12389.

Cohan, F. M., and A. A. Hoffmann. 1986. "Genetic divergence under uniform selection. II. Different responses to selection for knockdown resistance to ethanol among *Drosophila melanogaster* populations and their replicate lines." *Genetics* 114 (1): 145–64. http://www.genetics.org/content/114/1/145.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, et al. 2013. "Multiplex genome engineering using CRISPR/Cas systems." *Science* 339 (6121): 819–23. doi: 10.1126/science.1231143.

Conklin, E. G. 1929. "Problems of development." *American Naturalist* 63 (684): 5–36. doi: 10.1086/280235.

Cooley, L., R. Kelley, and A. Spradling. 1988. "Insertional mutagenesis of the Drosophila genome with single P elements." *Science* 239 (4844): 1121–28. doi: 10.1126/science.2830671.

Cooper, A. A., A. D. Gitler, A. Cashikar, C. M. Haynes, K. J. Hill, B. Bhullar, K. Liu, et al. 2006. "Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models." *Science* 313 (5785): 324–28. doi: 10.1126/science.1129462.

Cosens, D. J., and A. Manning. 1969. "Abnormal electroretinogram from a *Drosophila* mutant." *Nature* 224 (5216): 285–87. doi: 10.1038/224285a0.

Coulom, H., and S. Birman. 2004. "Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*." *J Neurosci* 24 (48): 10993–98. doi: 10.1523/jneurosci.2993-04.2004.

Crosby, M. A., L. S. Gramates, G. Dos Santos, B. B. Matthews, S. E. St Pierre, P. Zhou, A. J. Schroeder, et al. 2015. "Gene model annotations for *Drosophila melanogaster*: the rule-benders." *G3 (Bethesda)* 5 (8): 1737–49. doi: 10.1534/g3.115.018937.

Crow, J. F. 1954. "Analysis of a DDT-resistant strain of Drosophila." *Journal of Economic Entomology* 47:393–398. doi: 10.1093/jee/47.3.393.

Crow, J. F. 1957. "Genetics of insect resistance to chemicals." *Annual Review of Entomology* 2 (1): 227–246. doi: 10.1146/annurev.en.02.010157.001303.

Curran, C. H., and C. P. Alexander. 1934. *The families and genera of North American Diptera*. New York: C. H. Curran.

Curtin, J. A., E. Quint, V. Tsipouri, R. M. Arkell, B. Cattanach, A. J. Copp, D. J. Henderson, N. et al. 2003. "Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse." *Curr Biol* 13 (13): 1129–33. doi: 10.1016/S0960-9822(03)00374-9.

Damann, N., T. Voets, and B. Nilius. 2008. "TRPs in our senses." *Curr Biol* 18 (18): R880–89. doi: 10.1016/j.cub.2008.07.063.

Dambroise, E., L. Monnier, L. Ruisheng, H. Aguilaniu, J. S. Joly, H. Tricoire, and M. Rera. 2016. "Two phases of aging separated by the Smurf transition as a public path to death." *Sci Rep* 6:23523. doi: 10.1038/srep23523.

Das, G., S. Lin, and S. Waddell. 2016. "Remembering components of food in *Drosophila*." *Front Integr Neurosci* 10:4. doi: 10.3389/fnint.2016.00004.

Das, T., and R. Cagan. 2010. "*Drosophila* as a novel therapeutic discovery tool for thyroid cancer." *Thyroid* 20 (7): 689–95. doi: 10.1089/thy.2010.1637.

DasGupta, R., A. Kaykas, R. T. Moon, and N. Perrimon. 2005. "Functional genomic analysis of the Wnt-Wingless signaling pathway." *Science* 308 (5723): 826–33. doi: 10.1126/science.1109374.

Dävring, L. 1969. "The reaction of different *Drosophila* populations to treatment with pesticides." *Hereditas* 62 (3): 303–13. doi: 10.1111/j.1601-5223.1969.tb02240.x.

Dawson, A. G., P. Heidari, S. R. Gadagkar, M. J. Murray, and G. B. Call. 2013. "An airtight approach to the inebriometer: from construction to application with volatile anesthetics." *Fly (Austin)* 7 (2): 112–17. doi: 10.4161/fly.24142.

Dawson, T. M., H. S. Ko, and V. L. Dawson. 2010. "Genetic animal models of Parkinson's disease." *Neuron* 66 (5): 646–61. doi: 10.1016/j.neuron.2010.04.034.

de Beco, S., M. Ziosi, and L. A. Johnston. 2012. "New frontiers in cell competition." *Dev Dyn* 241 (5): 831–41. doi: 10.1002/dvdy.23783.

de la Cova, C., M. Abril, P. Bellosta, P. Gallant, and L. A. Johnston. 2004. "*Drosophila* Myc regulates organ size by inducing cell competition." *Cell* 117 (1): 107–16. doi: 10.1016/S0092-8674(04)00214-4.

Denell, R. E., and R. D. Frederick. 1983. "Homoeosis in *Drosophila*: a description of the Polycomb lethal syndrome." *Dev Biol* 97 (1): 34–47. doi: 10.1016/0012-1606(83)90061-1.

Devenport, D. 2016. "Tissue morphodynamics: translating planar polarity cues into polarized cell behaviors." *Semin Cell Dev Biol* 55:99–110. doi: 10.1016/j.semcdb.2016.03.012.

Devineni, A. V., and U. Heberlein. 2010. "Addiction-like behavior in *Drosophila*." *Communicative & Integrative Biology* 3 (4): 357–359. doi: 10.4161/cib.3.4.11885.

Dew-Budd, K., J. Jarnigan, and L. K. Reed. 2016. "Genetic and sex-specific transgenerational dffects of a high fat diet in *Drosophila melanogaster*." *PLoS One* 11 (8): e0160857. doi: 10.1371/journal.pone.0160857.

Di-Poi, N., J. I. Montoya-Burgos, H. Miller, O. Pourquie, M. C. Milinkovitch, and D. Duboule. 2010. "Changes in *Hox* genes' structure and function during the evolution of the squamate body plan." *Nature* 464 (7285): 99–103. doi: 10.1038/nature08789.

Dong, B., and S. Hayashi. 2015. "Shaping of biological tubes by mechanical interaction of cell and extracellular matrix." *Curr Opin Genet Dev* 32:129–34. doi: 10.1016/j.gde.2015.02.009.

Donner, A. L., and R. L. Maas. 2004. "Conservation and non-conservation of genetic pathways in eye specification." *Int J Dev Biol* 48 (8–9): 743–53. doi: 10.1387/ijdb.041877ad.

Doolittle, W. F., P. Fraser, M. B. Gerstein, B. R. Graveley, S. Henikoff, C. Huttenhower, A. Oshlack, et al. 2013. "Sixty years of genome biology." *Genome Biol* 14 (4): 113. doi: 10.1186/gb-2013-14-4-113.

Doroquez, D. B., and I. Rebay. 2006. "Signal integration during development: mechanisms of EGFR and Notch pathway function and cross-talk." *Crit Rev Biochem Mol Biol* 41 (6): 339–85. doi: 10.1080/10409230600914344.

dos Santos, G., A. J. Schroeder, J. L. Goodman, V. B. Strelets, M. A. Crosby, J. Thurmond, D. B. Emmert, W. M. Gelbart, and the FlyBase Consortium. 2015. "FlyBase: introduction of the *Drosophila melanogaster* Release 6 reference genome assembly and large-scale migration of genome annotations." *Nucleic Acids Res* 43 (Database issue): D690–97. doi: 10.1093/nar/gku1099.

Drayna, D. 2006. "Is our behavior written in our genes?" *N Engl J Med* 354 (1): 7–9. doi: 10.1056/NEJMp058215.

Drechsler, M., A. C. Schmidt, H. Meyer, and A. Paululat. 2013. "The conserved ADAMTS-like protein Lonely heart mediates matrix formation and cardiac tissue integrity." *PLoS Genet* 9 (7): e1003616. doi: 10.1371/journal.pgen.1003616.

Drosophila 12 Genomes Consortium. 2007. "Evolution of genes and genomes on the *Drosophila* phylogeny." *Nature* 450 (7167): 203–18. doi: 10.1038/nature06341.

Duncan, I. M. 1982. "Polycomblike: a gene that appears to be required for the normal expression of the bithorax and antennapedia gene complexes of *Drosophila melanogaster*." *Genetics* 102 (1): 49–70. http://www.genetics.org/content/102/1/49.

Dunn, L. C. 1934. *Heredity and variation: continuity and change in the living world*. University Series Highlights of Modern Knowledge: Genetics. New York: The University Society.

Dunne, A., N. A. Marshall, and K. H. Mills. 2011. "TLR based therapeutics." *Curr Opin Pharmacol* 11 (4): 404–11. doi: 10.1016/j.coph.2011.03.004.

Eaton, S. 1997. "Planar polarization of *Drosophila* and vertebrate epithelia." *Curr Opin Cell Biol* 9 (6): 860–66. doi: 10.1016/S0955-0674(97)80089-0.

Eberl, D. F., and G. Boekhoff-Falk. 2007. "Development of Johnston's organ in *Drosophila*." *Int J Dev Biol* 51 (6–7): 679–87. doi: 10.1387/ijdb.072364de.

Echalier, G. 1997. *Drosophila Cells in Culture*. San Diego: Academic Press.

Eddison, M., I. Le Roux, and J. Lewis. 2000. "Notch signaling in the development of the inner ear: lessons from *Drosophila*." *Proc Natl Acad Sci U S A* 97 (22): 11692–99. doi: 10.1073/pnas.97.22.11692.

Edwards, A. C., S. M. Rollmann, T. J. Morgan, and T. F. Mackay. 2006. "Quantitative genomics of aggressive behavior in *Drosophila melanogaster*." *PLoS Genet* 2 (9): e154. doi: 10.1371/journal.pgen.0020154.

Eiberg, H., J. Mohr, K. Schmiegelow, L. S. Nielsen, and R. Williamson. 1985. "Linkage relationships of paraoxonase (PON) with other markers: indication of PON-cystic fibrosis synteny." *Clin Genet* 28 (4): 265–71. doi: 10.1111/j.1399-0004.1985.tb00400.x.

Ekins, S., N. K. Litterman, R. J. Arnold, R. W. Burgess, J. S. Freundlich, S. J. Gray, J. J. Higgins, et al. 2015. "A brief review of recent Charcot-Marie-Tooth research and priorities." *F1000Res* 4:53. doi: 10.12688/f1000research.6160.1.

Evans, C. J., V. Hartenstein, and U. Banerjee. 2003. "Thicker than blood: conserved mechanisms in *Drosophila* and vertebrate hematopoiesis." *Dev Cell* 5 (5): 673–90. doi: 10.1016/S1534-5807(03)00335-6.

Fabre, J.-H.. 1915. *The life of the fly; with which are interspersed some chapters of autobiography*. Translated by A. Teixeira De Mattos. New York: Dodd, Mead and Company.

Fang, Y., and N. M. Bonini. 2015. "Hope on the (fruit) fly: the *Drosophila* wing paradigm of axon injury." *Neural Regen Res* 10 (2): 173–75. doi: 10.4103/1673-5374.152359.

Fanto, M., and H. McNeill. 2004. "Planar polarity from flies to vertebrates." *J Cell Sci* 117 (Pt 4): 527–33. doi: 10.1242/jcs.00973.

Feany, M. B., and W. W. Bender. 2000. "A *Drosophila* model of Parkinson's disease." *Nature* 404 (6776): 394–98. doi: 10.1038/35006074.

Fedeles, S., and A. R. Gallagher. 2013. "Cell polarity and cystic kidney disease." *Pediatr Nephrol* 28 (8): 1161–72. doi: 10.1007/s00467-012-2337-z.

Fernandez-Hernandez, I., E. Scheenaard, G. Pollarolo, and C. Gonzalez. 2016. "The translational relevance of *Drosophila* in drug discovery." *EMBO Rep* 17 (4): 471–472. doi: 10.15252/embr.201642080.

Ferrandon, D., J. L. Imler, C. Hetru, and J. A. Hoffmann. 2007. "The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections." *Nat Rev Immunol* 7 (11): 862–74. doi: 10.1038/nri2194.

Feske, S., Y. Gwack, M. Prakriya, S. Srikanth, S. H. Puppel, B. Tanasa, P. G. Hogan, R. S. Lewis, M. Daly, and A. Rao. 2006. "A mutation in Orai1 causes immune

deficiency by abrogating CRAC channel function." *Nature* 441 (7090): 179–85. doi: 10.1038/nature04702.

Fitch, K. R., G. K. Yasuda, K. N. Owens, and B. T. Wakimoto. 1998. "Paternal effects in *Drosophila*: implications for mechanisms of early development." *Curr Top Dev Biol* 38:1–34. http://doi.org/10.1016/S0070-2153(08)60243-4.

Flatt, T., L. L. Moroz, M. Tatar, and A. Heyland. 2006. "Comparing thyroid and insect hormone signaling." *Integr Comp Biol* 46 (6): 777–94. doi: 10.1093/icb/icl034.

The FlyBase Consortium. 1994. "FlyBase—the *Drosophila* database." *Nucleic Acids Res* 22 (17): 3456–58. doi: 10.1093/nar/24.1.53.

Forbes, A. J., H. Lin, P. W. Ingham, and A. C. Spradling. 1996. "*hedgehog* is required for the proliferation and specification of ovarian somatic cells prior to egg chamber formation in *Drosophila*." *Development* 122 (4): 1125–35. http://dev.biologists.org/content/122/4/1125.

Ford, C. E., J. L. Hamerton, D. W. Barnes, and J. F. Loutit. 1956. "Cytological identification of radiation-chimaeras." *Nature* 177 (4506): 452–54. doi: 10.1038/177452a0.

Ford, C. E., and H. S. Micklem. 1963. "The thymus and lymph-nodes in radiation chimaeras." *Lancet* 281 (7277): 359–62. doi: 10.1016/S0140-6736(63)91385-0.

Frasch, M. 2016. "Genome-wide approaches to *Drosophila* heart development." *J Cardiovasc Dev Dis* 3 (2). doi: 10.3390/jcdd3020020.

Freeman, A., E. Pranski, R. D. Miller, S. Radmard, D. Bernhard, H. A. Jinnah, R. Betarbet, D. B. Rye, and S. Sanyal. 2012. "Sleep fragmentation and motor restlessness in a *Drosophila* model of Restless Legs Syndrome." *Curr Biol* 22 (12): 1142–48. doi: 10.1016/j.cub.2012.04.027.

Freeman, E. G., and A. Dahanukar. 2015. "Molecular neurobiology of *Drosophila* taste." *Curr Opin Neurobiol* 34:140–48. doi: 10.1016/j.conb.2015.06.001.

Frolov, R. V., A. Bagati, B. Casino, and S. Singh. 2012. "Potassium channels in *Drosophila*: historical breakthroughs, significance, and perspectives." *J Neurogenet* 26 (3–4): 275–90. doi: 10.3109/01677063.2012.744990.

Gade, G., K. H. Hoffmann, and J. H. Spring. 1997. "Hormonal regulation in insects: facts, gaps, and future directions." *Physiol Rev* 77 (4): 963–1032. http://physrev.physiology.org/content/77/4/963.

Gall, J. G. 2015. "The origin of in situ hybridization—A personal history." *Methods*. doi: 10.1016/j.ymeth.2015.11.026.

Garcia-Bellido, A., and P. Ripoll. 1978. "The number of genes in *Drosophila melanogaster*." *Nature* 273 (5661): 399–400. doi: 10.1038/273399a0.

Gee, H. Y., F. Zhang, S. Ashraf, S. Kohl, C. E. Sadowski, V. Vega-Warner, W. Zhou, et al. 2015. "KANK deficiency leads to podocyte dysfunction and nephrotic syndrome." *J Clin Invest* 125 (6): 2375–84. doi: 10.1172/JCI79504.

Gelling, C. 2016. "The evolution of Dark-fly." *Genes to Genomes* (blog), *Genetics Society of America,* Feb. 4. https://genestogenomes.org/the-evolution-of-dark-fly/.

Gelman, A., M. Rawet-Slobodkin, and Z. Elazar. 2015. "Huntingtin facilitates selective autophagy." *Nat Cell Biol* 17 (3): 214–15. doi: 10.1038/ncb3125.

Gibbs, B. C., R. R. Damerla, E. K. Vladar, B. Chatterjee, Y. Wan, X. Liu, C. Cui, et al. 2016. "*Prickle1* mutation causes planar cell polarity and directional cell migration defects associated with cardiac outflow tract anomalies and other structural birth defects." *Biol Open* 5 (3): 323–35. doi: 10.1242/bio.015750.

Gladstone, M., B. Frederick, D. Zheng, A. Edwards, P. Yoon, S. Stickel, T. DeLaney, D. C. Chan, D. Raben, and T. T. Su. 2012. "A translation inhibitor identified in a *Drosophila* screen enhances the effect of ionizing radiation and taxol in mammalian models of cancer." *Dis Model Mech* 5 (3): 342–50. doi: 10.1242/dmm.008722.

Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, et al. 1996. "Life with 6000 genes." *Science* 274 (5287): 546, 563–67. doi: 10.1126/science.274.5287.546.

Gogna, R., K. Shee, and E. Moreno. 2015. "Cell competition during growth and regeneration." *Annu Rev Genet* 49:697–718. doi: 10.1146/annurev-genet-112414-055214.

Gold, K. S., and K. Bruckner. 2014. "*Drosophila* as a model for the two myeloid blood cell systems in vertebrates." *Exp Hematol* 42 (8): 717–27. doi: 10.1016/j.exphem.2014.06.002.

Goldman, I. L. 2002. "Raymond Pearl, smoking and longevity." *Genetics* 162 (3): 997–1001. http://www.genetics.org/content/162/3/997.

Golic, K. G., and S. Lindquist. 1989. "The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome." *Cell* 59 (3): 499–509. doi: 10.1016/0092-8674(89)90033-0.

Goriaux, C., E. Theron, E. Brasset, and C. Vaury. 2014. "History of the discovery of a master locus producing piRNAs: the flamenco/COM locus in *Drosophila melanogaster*." *Front Genet* 5:257. doi: 10.3389/fgene.2014.00257.

Graf, U., S. K. Abraham, J. Guzman-Rincon, and F. E. Wurgler. 1998. "Antigenotoxicity studies in *Drosophila melanogaster*." *Mutat Res* 402 (1–2): 203–9. doi: 10.1016/S0027-5107(97)00298-4.

Graf, U., F. E. Wurgler, A. J. Katz, H. Frei, H. Juon, C. B. Hall, and P. G. Kale. 1984. "Somatic mutation and recombination test in *Drosophila melanogaster*." *Environ Mutagen* 6 (2): 153–88. doi: 10.1002/em.2860060206.

Greenleaf, A. L., L. M. Borsett, P. F. Jiamachello, and D. E. Coulter. 1979. "Alpha-amanitin-resistant *D. melanogaster* with an altered RNA polymerase II." *Cell* 18 (3): 613–22. doi: 10.1016/0092-8674(79)90116-8.

Greenspan, L. J., M. de Cuevas, and E. Matunis. 2015. "Genetics of gonadal stem cell renewal." *Annu Rev Cell Dev Biol* 31:291–315. doi: 10.1146/annurev-cellbio-100913-013344.

Grimaldi, D., P. S. Ginsberg, L. Thayer, S. McEvey, M. Hauser, M. Turelli, and B. Brown. 2015. "Strange little flies in the big city: exotic flower-breeding drosophilidae (Diptera) in urban Los Angeles." *PLoS One* 10 (4): e0122575. doi: 10.1371/journal.pone.0122575.

Grochowski, C. M., K. M. Loomes, and N. B. Spinner. 2016. "Jagged1 (JAG1): structure, expression, and disease associations." *Gene* 576 (1 Pt 3): 381–84. doi: 10.1016/j.gene.2015.10.065.

Grossman, T. R., A. Gamliel, R. J. Wessells, O. Taghli-Lamallem, K. Jepsen, K. Ocorr, J. R. Korenberg, et al. 2011. "Over-expression of DSCAM and COL6A2 cooperatively generates congenital heart defects." *PLoS Genet* 7 (11): e1002344. doi: 10.1371/journal.pgen.1002344.

Grusche, F. A., H. E. Richardson, and K. F. Harvey. 2010. "Upstream regulation of the Hippo size control pathway." *Curr Biol* 20 (13): R574–82. doi: 10.1016/j.cub.2010.05.023.

Guarnieri, D. J., and U. Heberlein. 2003. "*Drosophila melanogaster*, a genetic model system for alcohol research." *Int Rev Neurobiol* 54:199–228. doi: 10.1016/S0074-7742(03)54006-5.

Gubb, D., and A. Garcia-Bellido. 1982. "A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*." *J Embryol Exp Morphol* 68:37–57. http://dev.biologists.org/content/68/1/37.

Gutierrez, E., D. Wiggins, B. Fielding, and A. P. Gould. 2007. "Specialized hepatocyte-like cells regulate *Drosophila* lipid metabolism." *Nature* 445 (7125): 275–80. doi: 10.1038/nature05382.

Hadorn, E. 1937. "Transplantation of gonads from lethal to normal larvae in *Drosophila melanogaster*." *Proceedings of the Society for Experimental Biology and Medicine* 36(5): 632–34. doi: 10.3181/00379727-36-9338P.

Hahn, I., M. Ronshaugen, N. Sanchez-Soriano, and A. Prokop. 2016. "Functional and Genetic Analysis of Spectraplakins in Drosophila." *Methods Enzymol* 569:373–405. http://doi.org/10.1016/bs.mie.2015.06.022.

Halder, G., P. Callaerts, and W. J. Gehring. 1995. "Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*." *Science* 267 (5205): 1788–92. http://doi.org/10.1126/science.7892602.

Han, B. W., and P. D. Zamore. 2014. "piRNAs." *Curr Biol* 24 (16): R730–33. doi: 10.1016/j.cub.2014.07.037.

Hanesch, U., K. F. Fischbach, and M. Heisenberg. 1989. "Neuronal architecture of the central complex in *Drosophila melanogaster*." *Cell and Tissue Research* 257 (2): 343–366. doi: 10.1007/BF00261838.

Hannah-Alava, A. 1958. "Morphology and chaetotaxy of the legs of *Drosophila melanogaster*." *Journal of Morphology* 103 (2): 281–310. doi: 10.1002/jmor.1051030205.

Hannah-Alava, A. 1969. "Localization of *Pc* and *Scx*." *Drosophila Information Service* 44:75–76.

Hanson, F. B. 1928. "The effect of X-rays in producing return gene mutations." *Science* 67 (1744): 562–63. doi: 10.1126/science.67.1744.562.

Hardy, J., and A. Singleton. 2009. "Genomewide association studies and human disease." *N Engl J Med* 360 (17): 1759–68. doi: 10.1056/NEJMra0808700.

Harel, T., W. H. Yoon, C. Garone, S. Gu, Z. Coban-Akdemir, M. K. Eldomery, J. E. Posey, et al. 2016. "Recurrent de novo and biallelic variation of ATAD3A, encoding a mitochondrial membrane protein, results in distinct neurological syndromes." *Am J Hum Genet* 99 (4): 831–45. doi: 10.1016/j.ajhg.2016.08.007.

Hariharan, I. K. 2015. "Organ size control: lessons from *Drosophila*." *Dev Cell* 34 (3): 255–65. doi: 10.1016/j.devcel.2015.07.012.

Harvey, K. F., C. M. Pfleger, and I. K. Hariharan. 2003. "The *Drosophila* Mst ortholog, *hippo*, restricts growth and cell proliferation and promotes apoptosis." *Cell* 114 (4): 457–67. doi: 10.1016/S0092-8674(03)00557-9.

Haynie, J. L. 1983. "The maternal and zygotic roles of the gene *Polycomb* in embryonic determination in *Drosophila melanogaster*." *Dev Biol* 100 (2): 399–411. doi: 10.1016/0012-1606(83)90234-8.

Hecht, J. T., D. Hogue, L. C. Strong, M. F. Hansen, S. H. Blanton, and M. Wagner. 1995. "Hereditary multiple exostosis and chondrosarcoma: linkage to chromosome II and loss of heterozygosity for EXT-linked markers on chromosomes II and 8." *Am J Hum Genet* 56 (5): 1125–31. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1801450/.

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson. 2008. "*Wolbachia* and virus protection in insects." *Science* 322 (5902): 702. doi: 10.1126/science.1162418.

Hemmi, H., T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda, and S. Akira. 2002. "Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway." *Nat Immunol* 3 (2): 196–200. doi: 10.1038/ni758.

Henderson, D. J., and B. Chaudhry. 2011. "Getting to the heart of planar cell polarity signaling." *Birth Defects Res A Clin Mol Teratol* 91 (6): 460–67. doi: 10.1002/bdra.20792.

Henikoff, S. 2015. "The genetic map enters its second century." *Genetics* 200:671–74. doi: 10.1534/genetics.115.178434.

Herman, M. M., J. Miquel, and M. Johnson. 1971. "Insect brain as a model for the study of aging. Age-related changes in *Drosophila melanogaster*." *Acta Neuropathol* 19 (3): 167–83. doi: 10.1007/BF00684595.

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren. 2008. "How many species are infected with *Wolbachia*?—A statistical analysis of current data." *FEMS Microbiol Lett* 281 (2): 215–20. doi: 10.1111/j.1574-6968.2008.01110.x.

Hisahara, S., and S. Shimohama. 2010. "Toxin-induced and genetic animal models of Parkinson's disease." *Parkinsons Dis* 2011:951709. doi: 10.4061/2011/951709.

Hoffman-Falk, H., P. Einat, B. Z. Shilo, and F. M. Hoffmann. 1983. "*Drosophila melanogaster* DNA clones homologous to vertebrate oncogenes: evidence for a common ancestor to the *src* and *abl* cellular genes." *Cell* 32 (2): 589–98. doi: 10.1016/0092-8674(83)90478-6.

Hoffmann, A. A., B. L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P. H. Johnson, F. Muzzi, M. Greenfield, et al. 2011. "Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission." *Nature* 476 (7361): 454–57. doi: 10.1038/nature10356.

Hoge, M. A. 1915. "Another gene in the fourth chromosome of *Drosophila*." *American Naturalist* 49:47–49. http://www.jstor.org/stable/2456099.

Holladay, C., M. Kulkarni, W. Minor, and A. Pandit. 2012. "Gene therapy." In *Tissue engineering: principles and practices,* edited by J. P. Fisher, A. G. Mikos, J. D. Bronzino and D. R. Peterson. Boca Raton, FL: CRC Press.

Hollfelder, D., M. Frasch, and I. Reim. 2014. "Distinct functions of the laminin beta LN domain and collagen IV during cardiac extracellular matrix formation and stabilization of alary muscle attachments revealed by EMS mutagenesis in *Drosophila*." *BMC Dev Biol* 14:26. doi: 10.1186/1471-213X-14-26.

Homem, C. C., and J. A. Knoblich. 2012. "*Drosophila* neuroblasts: a model for stem cell biology." *Development* 139 (23): 4297–310. doi: 10.1242/dev.080515.

Homyk, T., Jr., J. Szidonya, and D. T. Suzuki. 1980. "Behavioral mutants of *Drosophila melanogaster*. III. Isolation and mapping of mutations by direct visual observations of behavioral phenotypes." *Mol Gen Genet* 177 (4): 553–65. doi: 10.1007/BF00272663.

Housden, B. E., A. J. Valvezan, C. Kelley, R. Sopko, Y. Hu, C. Roesel, S. Lin, et al. 2015. "Identification of potential drug targets for tuberous sclerosis complex by synthetic screens combining CRISPR-based knockouts with RNAi." *Sci Signal* 8 (393): rs9. doi: 10.1126/scisignal.aab3729.

Hovhannisyan, A., M. Matz, and R. Gebhardt. 2009. "From teratogens to potential therapeutics: natural inhibitors of the Hedgehog signaling network come of age." *Planta Med* 75 (13): 1371–80. doi: 10.1055/s-0029-1185979.

Howard, L. O. 1901. *Mosquitoes; how they live; how they carry disease; how they are classified; how they may be destroyed.* New York: McClure, Phillips.

Hsieh, C. H., A. Shaltouki, A. E. Gonzalez, A. Bettencourt da Cruz, L. F. Burbulla, E. St Lawrence, B. Schule, D. Krainc, T. D. Palmer, and X. Wang. 2016. "Functional impairment in Miro degradation and mitophagy is a shared feature in familial and sporadic Parkinson's disease." *Cell Stem Cell.* 19(6): 709–724. doi: 10.1016/j.stem.2016.08.002.

Huang, X., Y. He, A. M. Dubuc, R. Hashizume, W. Zhang, J. Reimand, H. Yang, et al. 2015. "EAG2 potassium channel with evolutionarily conserved function as a brain tumor target." *Nat Neurosci* 18 (9): 1236–46. doi: 10.1038/nn.4088.

Hughes, C. L., and T. C. Kaufman. 2002. "Exploring the myriapod body plan: expression patterns of the ten Hox genes in a centipede." *Development* 129 (5): 1225–38. http://dev.biologists.org/content/129/5/1225.

Ibarra-Laclette, E., E. Lyons, G. Hernandez-Guzman, C. A. Perez-Torres, L. Carretero-Paulet, T. H. Chang, T. Lan, et al. 2013. "Architecture and evolution of a minute plant genome." *Nature* 498 (7452): 94–98. doi: 10.1038/nature12132.

Ingerson-Mahar, M., A. Briegel, J. N. Werner, G. J. Jensen, and Z. Gitai. 2010. "The metabolic enzyme CTP synthase forms cytoskeletal filaments." *Nat Cell Biol* 12 (8): 739–46. doi: 10.1038/ncb2087.

International Glossina Genome Initiative. 2014. "Genome sequence of the tsetse fly (*Glossina morsitans*): vector of African trypanosomiasis." *Science* 344 (6182): 380–86. doi: 10.1126/science.1249656.

Ishikawa, Y., and A. Kamikouchi. 2015. "Auditory system of fruit flies." *Hear Res.* 338:1–8. doi: 10.1016/j.heares.2015.10.017.

Ito, S., T. Ueda, A. Ueno, H. Nakagawa, H. Taniguchi, N. Kayukawa, and T. Miki. 2014. "A genetic screen in *Drosophila* for regulators of human prostate cancer progression." *Biochem Biophys Res Commun* 451 (4): 548–55. doi: 10.1016/j.bbrc.2014.08.015.

Jackson, G. R., I. Salecker, X. Dong, X. Yao, N. Arnheim, P. W. Faber, M. E. MacDonald, and S. L. Zipursky. 1998. "Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons." *Neuron* 21 (3): 633–42. doi: 10.1016/S0896-6273(00)80573-5.

Jacquemont, S., A. Curie, V. des Portes, M. G. Torrioli, E. Berry-Kravis, R. J. Hagerman, F. J. Ramos, et al. 2011. "Epigenetic modification of the *FMR1* gene in Fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056." *Sci Transl Med* 3 (64): 64ra1. doi: 10.1126/scitranslmed.3001708.

Jan, L. Y., and Y. N. Jan. 1997. "Voltage-gated and inwardly rectifying potassium channels." *J Physiol* 505 (Pt2): 267–82. doi: 10.1111/j.1469-7793.1997.267bb.x.

Jansen R., J. D. Embden, W. Gaastra, and L. M. Schouls. 2002. "Identification of genes that are associated with DNA repeats in prokaryotes." *Mol Microbiol.* 43 (6): 1565–75. doi: 10.1046/j.1365-2958.2002.02839.x.

Jeffries, C. L., and T. Walker. 2015. "The potential use of *Wolbachia*-based mosquito biocontrol strategies for Japanese encephalitis." *PLoS Negl Trop Dis* 9 (6): e0003576. doi: 10.1371/journal.pntd.0003576.

Jellinger, K. A. 2013. "The relevance of metals in the pathophysiology of neurodegeneration, pathological considerations." *Int Rev Neurobiol* 110:1–47. doi: 10.1016/B978-0-12-410502-7.00002-8.

Jenett, A., G. M. Rubin, T. T. Ngo, D. Shepherd, C. Murphy, H. Dionne, B. D. Pfeiffer, et al. 2012. "A GAL4-driver line resource for *Drosophila* neurobiology." *Cell Rep* 2 (4): 991–1001. doi: 10.1016/j.celrep. 2012.09.011.

Jiang, H., and B. A. Edgar. 2011. "Intestinal stem cells in the adult *Drosophila* midgut." *Exp Cell Res* 317 (19): 2780–88. doi: 10.1016/j.yexcr.2011.07.020.

Jin, S., S. Martinek, W. S. Joo, J. R. Wortman, N. Mirkovic, A. Sali, M. D. Yandell, N. P. Pavletich, M. W. Young, and A. J. Levine. 2000. "Identification and characterization of a p53 homologue in *Drosophila melanogaster*." *Proc Natl Acad Sci U S A* 97 (13): 7301–6. doi: 10.1073/pnas.97.13.7301.

Jin, Z., A. S. Flynt, and E. C. Lai. 2013. "*Drosophila piwi* mutants exhibit germline stem cell tumors that are sustained by elevated Dpp signaling." *Curr Biol* 23 (15): 1442–48. doi: 10.1016/j.cub.2013.06.021.

Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, and E. Charpentier. 2012. "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." *Science* 337 (6096): 816–21. doi: 10.1126/science.1225829.

Johnson, R., and G. Halder. 2014. "The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment." *Nat Rev Drug Discov* 13 (1): 63–79. doi: 10.1038/nrd4161.

Johnson, R. L., A. L. Rothman, J. Xie, L. V. Goodrich, J. W. Bare, J. M. Bonifas, A. G. Quinn, et al. 1996. "Human homolog of *patched*, a candidate gene for the basal cell nevus syndrome." *Science* 272 (5268): 1668–71. doi: 10.1126/science.272.5268.1668.

Johnston, L. A., D. A. Prober, B. A. Edgar, R. N. Eisenman, and P. Gallant. 1999. "*Drosophila myc* regulates cellular growth during development." *Cell* 98 (6): 779–90. doi: 10.1016/S0092-8674(00)81512-3.

Jousset, F. X., M. Bergoin, and B. Revet. 1977. "Characterization of the *Drosophila* C virus." *J Gen Virol* 34 (2): 269–83. doi: 10.1099/0022-1317-34-2-269.

Juraeva, D., J. Treutlein, H. Scholz, J. Frank, F. Degenhardt, S. Cichon, M. Ridinger, et al. 2015. "XRCC5 as a risk gene for alcohol dependence: evidence from a genome-wide gene-set-based analysis and follow-up studies in *Drosophila* and humans." *Neuropsychopharmacology* 40 (2): 361–71. doi: 10.1038/npp.2014.178.

Justice, R. W., O. Zilian, D. F. Woods, M. Noll, and P. J. Bryant. 1995. "The *Drosophila* tumor suppressor gene *warts* encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation." *Genes Dev* 9 (5): 534–46. doi: 10.1101/gad.9.5.534.

Kacsoh, B. Z., J. Bozler, M. Ramaswami, and G. Bosco. 2015. "Social communication of predator-induced changes in *Drosophila* behavior and germ line physiology." *Elife* 4: e07423. doi: 10.7554/eLife.07423.

Kacsoh, B. Z., Z. R. Lynch, N. T. Mortimer, and T. A. Schlenke. 2013. "Fruit flies medicate offspring after seeing parasites." *Science* 339 (6122): 947–50. doi: 10.1126/science.1229625.

Kain, J. S., C. Stokes, and B. L. de Bivort. 2012. "Phototactic personality in fruit flies and its suppression by serotonin and *white*." *Proc Natl Acad Sci U S A* 109 (48): 19834–39. doi: 10.1073/pnas.1211988109.

Kanatsu-Shinohara, M., K. Inoue, S. Takashima, M. Takehashi, N. Ogonuki, H. Morimoto, T. Nagasawa, A. Ogura, and T. Shinohara. 2012. "Reconstitution

of mouse spermatogonial stem cell niches in culture." *Cell Stem Cell* 11 (4): 567–78. doi: 10.1016/j.stem.2012.06.011.

Kango-Singh, M., R. Nolo, C. Tao, P. Verstreken, P. R. Hiesinger, H. J. Bellen, and G. Halder. 2002. "Shar-pei mediates cell proliferation arrest during imaginal disc growth in *Drosophila*." *Development* 129 (24): 5719–30. doi: 10.1242/dev.00168.

Kaplan, W. D., and W. E. Trout, 3rd. 1969. "The behavior of four neurological mutants of *Drosophila*." *Genetics* 61 (2): 399–409. http://www.genetics.org/content/61/2/399.

Kasuya, J., H. Ishimoto, and T. Kitamoto. 2009. "Neuronal mechanisms of learning and memory revealed by spatial and temporal suppression of neurotransmission using *shibire*, a temperature-sensitive dynamin mutant gene in *Drosophila melanogaster*." *Front Mol Neurosci* 2:11. doi: 10.3389/neuro.02.011.2009.

Katzenberger, R. J., B. Ganetzky, and D. A. Wassarman. 2015. "The gut reaction to traumatic brain injury." *Fly (Austin)* 9 (2): 68–74. doi: 10.1080/19336934.2015.1085623.

Katzenberger, R. J., C. A. Loewen, R. T. Bockstruck, M. A. Woods, B. Ganetzky, and D. A. Wassarman. 2015. "A method to inflict closed head traumatic brain injury in *Drosophila*." *J Vis Exp* (100): e52905. doi: 10.3791/52905.

Katzenberger, R. J., C. A. Loewen, D. R. Wassarman, A. J. Petersen, B. Ganetzky, and D. A. Wassarman. 2013. "A *Drosophila* model of closed head traumatic brain injury." *Proc Natl Acad Sci U S A* 110 (44): E4152–59. doi: 10.1073/pnas.1316895110.

Kaufman, T. C. 2017. "A short history and description of *Drosophila melanogaster* classical genetics: chromosome aberrations, forward genetic screens, and the nature of mutations." *Genetics* 206 (2): 665–689. doi: 10.1534/genetics.117.199950.

Kaun, K. R., A. V. Devineni, and U. Heberlein. 2012. "*Drosophila melanogaster* as a model to study drug addiction." *Hum Genet* 131 (6): 959–75. doi: 10.1007/s00439-012-1146-6.

Keebaugh, E. S., and T. A. Schlenke. 2014. "Insights from natural host-parasite interactions: the *Drosophila* model." *Dev Comp Immunol* 42 (1): 111–23. doi: 10.1016/j.dci.2013.06.001.

Kennerdell, J. R., and R. W. Carthew. 2000. "Heritable gene silencing in Drosophila using double-stranded RNA." *Nat Biotechnol* 18 (8): 896–98. doi: 10.1038/78531.

Kibar, Z., S. Salem, C. M. Bosoi, E. Pauwels, P. De Marco, E. Merello, A. G. Bassuk, V. Capra, and P. Gros. 2011. "Contribution of VANGL2 mutations to isolated neural tube defects." *Clin Genet* 80 (1): 76–82. doi: 10.1111/j.1399-0004.2010.01515.x.

Kibar, Z., E. Torban, J. R. McDearmid, A. Reynolds, J. Berghout, M. Mathieu, I. Kirillova, et al. 2007. "Mutations in VANGL1 associated with neural-tube defects." *N Engl J Med* 356 (14): 1432–37. doi: 10.1056/NEJMoa060651.

Kibar, Z., K. J. Vogan, N. Groulx, M. J. Justice, D. A. Underhill, and P. Gros. 2001. "Ltap, a mammalian homolog of *Drosophila* Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail." *Nat Genet* 28 (3): 251–55. doi: 10.1038/90081.

Kiger, A. A., B. Baum, S. Jones, M. R. Jones, A. Coulson, C. Echeverri, and N. Perrimon. 2003. "A functional genomic analysis of cell morphology using RNA interference." *J Biol* 2 (4): 27. doi: 10.1186/1475-4924-2-27.

Kiger, A. A., D. L. Jones, C. Schulz, M. B. Rogers, and M. T. Fuller. 2001. "Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue." *Science* 294 (5551): 2542–45. doi: 10.1126/science.1066707.

Kilbey, B. J., D. J. MacDonald, C. Auerbach, F. H. Sobels, and E. W. Vogel. 1981. "The use of *Drosophila melanogaster* in tests for environmental mutagens." *Mutat Res* 85 (3): 141–46. doi: 10.1016/0165-1161(81)90029-7.

King, L. B., M. Koch, K. R. Murphy, Y. Velazquez, W. W. Ja, and S. M. Tomchik. 2016. "Neurofibromin loss of function drives excessive grooming in *Drosophila*." *G3 (Bethesda)* 6 (4): 1083–93. doi: 10.1534/g3.115.026484.

Kitada, T., S. Asakawa, N. Hattori, H. Matsumine, Y. Yamamura, S. Minoshima, M. Yokochi, Y. Mizuno, and N. Shimizu. 1998. "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism." *Nature* 392 (6676): 605–8. doi: 10.1038/33416.

Kitamoto, T. 2001. "Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive shibire allele in defined neurons." *J Neurobiol* 47 (2): 81–92. doi: 10.1002/neu.1018.

Koana, T., M. O. Okada, M. Ikehata, and M. Nakagawa. 1997. "Increase in the mitotic recombination frequency in *Drosophila melanogaster* by magnetic field exposure and its suppression by vitamin E supplement." *Mutat Res* 373 (1): 55–60. doi: 10.1016/S0027-5107(96)00188-1.

Kohler, Robert E. 1994. *Lords of the fly: Drosophila genetics and the experimental life.* Chicago: University of Chicago Press.

Kolch, W., A. Kotwaliwale, K. Vass, and P. Janosch. 2002. "The role of Raf kinases in malignant transformation." *Expert Rev Mol Med* 4 (8): 1–18. doi: 10.1017/S1462399402004386.

Kongsuwan, K., Q. Yu, A. Vincent, M. C. Frisardi, M. Rosbash, J. A. Lengyel, and J. Merriam. 1985. "A *Drosophila Minute* gene encodes a ribosomal protein." *Nature* 317 (6037): 555–58. doi: 10.1038/317555a0.

Konopka, R. J., and S. Benzer. 1971. "Clock mutants of *Drosophila melanogaster*." *Proc Natl Acad Sci U S A* 68 (9): 2112–16. http://www.pnas.org/content/68/9/2112.

Korbel, J. O., T. Tirosh-Wagner, A. E. Urban, X. N. Chen, M. Kasowski, L. Dai, F. Grubert, et al. 2009. "The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies." *Proc Natl Acad Sci U S A* 106 (29): 12031–36. doi: 10.1073/pnas.0813248106.

Koundakjian, E. J., D. M. Cowan, R. W. Hardy, and A. H. Becker. 2004. "The Zuker collection: a resource for the analysis of autosomal gene function in *Drosophila melanogaster*." *Genetics* 167 (1): 203–6. doi: 10.1534/genetics.167.1.203.

Kraaijeveld, A. R., and H. C. Godfray. 2009. "Evolution of host resistance and parasitoid counter-resistance." *Adv Parasitol* 70:257–80. doi: 10.1016/S0065-308X(09)70010-7.

Krstic, D., W. Boll, and M. Noll. 2013. "Influence of the White locus on the courtship behavior of *Drosophila* males." *PLoS One* 8 (10): e77904. doi: 10.1371/journal.pone.0077904.

Kumar, D. K., S. H. Choi, K. J. Washicosky, W. A. Eimer, S. Tucker, J. Ghofrani, A. Lefkowitz, et al. 2016. "Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease." *Sci Transl Med* 8 (340): 340ra72. doi: 10.1126/scitranslmed.aaf1059.

Kwon, Y., A. Vinayagam, X. Sun, N. Dephoure, S. P. Gygi, P. Hong, and N. Perrimon. 2013. "The Hippo signaling pathway interactome." *Science* 342 (6159): 737–40. doi: 10.1126/science.1243971.

Lack, J. B., C. M. Cardeno, M. W. Crepeau, W. Taylor, R. B. Corbett-Detig, K. A. Stevens, C. H. Langley, and J. E. Pool. 2015. "The *Drosophila* genome nexus: a population genomic resource of 623 *Drosophila melanogaster* genomes, including 197 from a single ancestral range population." *Genetics* 199 (4): 1229–41. doi: 10.1534/genetics.115.174664.

Lack, J. B., A. Yassin, Q. D. Sprengelmeyer, E. J. Johanning, J. R. David, and J. E. Pool. 2016. "Life history evolution and cellular mechanisms associated with increased size in high-altitude *Drosophila*." *Ecol Evol* 6 (16): 5893–906. doi: 10.1002/ece3.2327.

Lack, J. B., J. D. Lange, A. B. Tang, R. B. Corbett-Detig, and J. E. Pool. 2016. "A thousand fly genomes: an expanded *Drosophila* genome nexus." *bioRxiv.* doi: 10.1101/063537.

Lauth, M., A. Bergstrom, T. Shimokawa, and R. Toftgard. 2007. "Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists." *Proc Natl Acad Sci U S A* 104 (20): 8455–60. doi: 10.1073/pnas.0609699104.

Lawrence, P. A. 1966. "Gradients in the insect segment: the orientation of hairs in the milkweed bug *Oncopeltus fasciatus*." *Journal of Experimental Biology* 44 (3): 607. http://jeb.biologists.org/content/44/3/607.

Lawrence, P. A. 1992. *The making of a fly: the genetics of animal design*. Oxford; Boston: Blackwell Scientific Publications.

Lawrence, P. A., and J. Casal. 2013. "The mechanisms of planar cell polarity, growth and the Hippo pathway: some known unknowns." *Dev Biol* 377 (1): 1–8. doi: 10.1016/j.ydbio.2013.01.030.

Lawrence, P. A., J. Casal, and G. Struhl. 2004. "Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*." *Development* 131 (19): 4651–64. doi: 10.1242/dev.01351.

Le Mouellic, H., Y. Lallemand, and P. Brulet. 1992. "Homeosis in the mouse induced by a null mutation in the *Hox-3.1* gene." *Cell* 69 (2): 251–64. doi: 10.1016/0092-8674(92)90406-3.

Lee, H. G., Y. C. Kim, J. S. Dunning, and K. A. Han. 2008. "Recurring ethanol exposure induces disinhibited courtship in *Drosophila*." *PLoS One* 3 (1): e1391. doi: 10.1371/journal.pone.0001391.

Leibovitch, B. A., D. B. Campbell, K. S. Krishnan, and H. A. Nash. 1995. "Mutations that affect ion channels change the sensitivity of *Drosophila melanogaster* to volatile anesthetics." *J Neurogenet* 10 (1): 1–13. doi: 10.3109/01677069509083455.

Lemaitre, B., and I. Miguel-Aliaga. 2013. "The digestive tract of *Drosophila melanogaster*." *Annu Rev Genet* 47:377–404. doi: 10.1146/annurev-genet-111212-133343.

Lenz, S., P. Karsten, J. B. Schulz, and A. Voigt. 2013. "*Drosophila* as a screening tool to study human neurodegenerative diseases." *J Neurochem* 127 (4): 453–60. doi: 10.1111/jnc.12446.

Lessa, F. C., Y. Mu, W. M. Bamberg, Z. G. Beldavs, G. K. Dumyati, J. R. Dunn, M. M. Farley, et al. 2015. "Burden of *Clostridium difficile* infection in the United States." *N Engl J Med* 372 (9): 825–34. doi: 10.1056/NEJMoa1408913.

Lewis, E. B. 1956. "New mutants report." *Drosophila Information Service* 30:76–77.

Lewis, E. B. 1978. "A gene complex controlling segmentation in *Drosophila*." *Nature* 276 (5688): 565–70. doi: 10.1038/276565a0.

Lewis, E. B. 1982. "Control of body segment differentiation in *Drosophila* by the *bithorax* gene complex." *Prog Clin Biol Res* 85 Pt A:269–88.

Lewis, E. B. 1994. "Homeosis: the first 100 years." *Trends Genet* 10 (10): 341–43. doi: 10.1016/0168-9525(94)90117-1.

Lewis, E. B., and Bacher, F. 1968. "Method of feeding ethyl methane sulfonate (EMS) to *Drosophila* males." *Drosophila Information Service* 43:193.

Lewis, P. H. 1947. "New mutants report." *Drosophila Information Service* 21:69.

Li, J., W. Zhang, Z. Guo, S. Wu, L. Y. Jan, and Y. N. Jan. 2016. "A defensive kicking behavior in response to mechanical stimuli mediated by *Drosophila* wing margin bristles." *J Neurosci* 36 (44): 11275–82. doi: 10.1523/jneurosci.1416-16.2016.

Li, T., N. Giagtzoglou, D. F. Eberl, S. N. Jaiswal, T. Cai, D. Godt, A. K. Groves, and H. J. Bellen. 2016. "The E3 ligase Ubr3 regulates Usher syndrome and *MYH9* disorder proteins in the auditory organs of *Drosophila* and mammals." *Elife* 5:e15258. doi: 10.7554/eLife.15258.

Liao, T. S., G. B. Call, P. Guptan, A. Cespedes, J. Marshall, K. Yackle, E. Owusu-Ansah, et al. 2006. "An efficient genetic screen in *Drosophila* to identify nuclear-encoded genes with mitochondrial function." *Genetics* 174 (1): 525–33. doi: 10.1534/genetics.106.061705.

Lima, S. Q., and G. Miesenböck. 2005. "Remote control of behavior through genetically targeted photostimulation of neurons." *Cell* 121 (1): 141–52. doi: 10.1016/j.cell.2005.02.004.

Lin, H. 2002. "The stem-cell niche theory: lessons from flies." *Nat Rev Genet* 3 (12): 931–40. doi: 10.1038/nrg952.

Lin, H., and A. C. Spradling. 1993. "Germline stem cell division and egg chamber development in transplanted *Drosophila* germaria." *Dev Biol* 159 (1): 140–52. doi: 10.1006/dbio.1993.1228.

Lin, S., B. Ewen-Campen, X. Ni, B. E. Housden, and N. Perrimon. 2015. "In vivo transcriptional activation using CRISPR/Cas9 in *Drosophila*." *Genetics* 201 (2): 433–42. doi: 10.1534/genetics.115.181065.

Lindsley, D. L., E. H. Grell, and C. B. Bridges. 1967. *Genetic variations of Drosophila melanogaster.* Carnegie Institution of Washington Publication. Washington, DC: Carnegie Intstitution of Washington.

Lindsley, D. L. and G. G. Zimm. 1992. *The genome of Drosophila melanogaster.* San Diego: Academic Press.

Lipshitz, H. D. 2005. "From fruit flies to fallout: Ed Lewis and his science." *Dev Dyn* 232 (3): 529–46. doi: 10.1002/dvdy.20332.

Liu, J. L. 2010. "Intracellular compartmentation of CTP synthase in *Drosophila*." *J Genet Genomics* 37 (5): 281–96. doi: 10.1016/S1673-8527(09)60046-1.

Liu, L., Y. Li, R. Wang, C. Yin, Q. Dong, H. Hing, C. Kim, and M. J. Welsh. 2007. "*Drosophila* hygrosensation requires the TRP channels *water witch* and *nanchung*." *Nature* 450 (7167): 294–98. doi: 10.1038/nature06223.

Lu, X., and C. W. Sipe. 2016. "Developmental regulation of planar cell polarity and hair-bundle morphogenesis in auditory hair cells: lessons from human and mouse genetics." *Wiley Interdiscip Rev Dev Biol* 5 (1): 85–101. doi: 10.1002/wdev.202.

Lum, L., S. Yao, B. Mozer, A. Rovescalli, D. Von Kessler, M. Nirenberg, and P. A. Beachy. 2003. "Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells." *Science* 299 (5615): 2039–45. doi: 10.1126/science.1081403.

Lumpkin, E. A., K. L. Marshall, and A. M. Nelson. 2010. "The cell biology of touch." *J Cell Biol* 191 (2): 237–48. doi: 10.1083/jcb.201006074.

Mackay, T. F., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles, D. Zhu, S. Casillas, et al. 2012. "The *Drosophila melanogaster* Genetic Reference Panel." *Nature* 482 (7384): 173–78. doi: 10.1038/nature10811.

Madabattula, S. T., J. C. Strautman, A. M. Bysice, J. A. O'Sullivan, A. Androschuk, C. Rosenfelt, K. Doucet, G. Rouleau, and F. Bolduc. 2015. "Quantitative analysis of climbing defects in a *Drosophila* model of neurodegenerative disorders." *J Vis Exp* (100): e52741. doi: 10.3791/52741.

Maheshwari, M., S. Bhutani, A. Das, R. Mukherjee, A. Sharma, Y. Kino, N. Nukina, and N. R. Jana. 2014. "Dexamethasone induces heat shock response and slows

down disease progression in mouse and fly models of Huntington's disease." *Hum Mol Genet* 23 (10): 2737–51. doi: 10.1093/hmg/ddt667.

Maheshwari, P., and G. D. Eslick. 2015. "Bacterial infection and Alzheimer's disease: a meta-analysis." *J Alzheimers Dis* 43 (3): 957–66. doi: 10.3233/JAD-140621.

Mali, P., L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville, and G. M. Church. 2013. "RNA-guided human genome engineering via Cas9." *Science* 339 (6121): 823–26. doi: 10.1126/science.1232033.

Marcos, R., and E. R. Carmona. 2013. "The wing-spot and the comet tests as useful assays detecting genotoxicity in *Drosophila*." *Methods Mol Biol* 1044:417–27. doi: 10.1007/978-1-62703-529-3_23.

Mark, G. E., R. J. MacIntyre, M. E. Digan, L. Ambrosio, and N. Perrimon. 1987. "*Drosophila melanogaster* homologs of the raf oncogene." *Mol Cell Biol* 7 (6): 2134–40. doi: 10.1128/MCB.7.6.2134.

Markstein, M., S. Dettorre, J. Cho, R. A. Neumüller, S. Craig-Muller, and N. Perrimon. 2014. "Systematic screen of chemotherapeutics in *Drosophila* stem cell tumors." *Proc Natl Acad Sci U S A* 111 (12): 4530–35. doi: 10.1073/pnas.1401160111.

Marshak-Rothstein, A. 2006. "Toll-like receptors in systemic autoimmune disease." *Nat Rev Immunol* 6 (11): 823–35. doi: 10.1038/nri1957.

Marshall, S. A. 2006. *Insects: their natural history and diversity: with a photographic guide to insects of eastern North America*. Buffalo, NY: Firefly Books.

Martin, F., T. Boto, C. Gomez-Diaz, and E. Alcorta. 2013. "Elements of olfactory reception in adult *Drosophila melanogaster*." *Anat Rec (Hoboken)* 296 (9): 1477–88. doi: 10.1002/ar.22747.

Martinelli, G., V. G. Oehler, C. Papayannidis, R. Courtney, M. N. Shaik, X. Zhang, A. O'Connell, et al. 2015. "Treatment with PF-04449913, an oral smoothened antagonist, in patients with myeloid malignancies: a phase 1 safety and pharmacokinetics study." *Lancet Haematology* 2 (8): e339-e346. doi: 10.1016/S2352-3026(15)00096-4.

Mathews, K. W., M. Cavegn, and M. Zwicky. 2017. "Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene *Myc* and by the sex-determining gene *tra* in *Drosophila*." *Genetics* 205 (3): 1215–28. doi: 10.1534/genetics.116.192260.

Mathieson, I., I. Lazaridis, N. Rohland, S. Mallick, N. Patterson, S. A. Roodenberg, E. Harney, et al. 2015. "Genome-wide patterns of selection in 230 ancient Eurasians." *Nature* 528 (7583): 499–503. doi: 10.1038/nature16152.

Matthews, B. B., G. dos Santos, M. A. Crosby, D. B. Emmert, S. E. St Pierre, L. S. Gramates, P. Zhou, et al. 2015. "Gene model annotations for *Drosophila melanogaster*: impact of high-throughput data." *G3 (Bethesda)* 5 (8): 1721–36. doi: 10.1534/g3.115.018929.

McBride, S. M., C. H. Choi, Y. Wang, D. Liebelt, E. Braunstein, D. Ferreiro, A. Sehgal, et al. 2005. "Pharmacological rescue of synaptic plasticity, courtship

behavior, and mushroom body defects in a *Drosophila* model of fragile X syndrome." *Neuron* 45 (5): 753–64. doi: 10.1016/j.neuron.2005.01.038.

McGinnis, W. 1994. "A century of homeosis, a decade of homeoboxes." *Genetics* 137 (3): 607–11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1206020/.

McGinnis, W., R. L. Garber, J. Wirz, A. Kuroiwa, and W. J. Gehring. 1984. "A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans." *Cell* 37 (2): 403–8. doi: 10.1016/0092-8674(84)90370-2.

McGinnis, W., M. S. Levine, E. Hafen, A. Kuroiwa, and W. J. Gehring. 1984. "A conserved DNA sequence in homoeotic genes of the *Drosophila* Antennapedia and bithorax complexes." *Nature* 308 (5958): 428–33. doi: 10.1038/308428a0.

Meister, M., and M. Lagueux. 2003. "*Drosophila* blood cells." *Cell Microbiol* 5 (9): 573–80. doi: 10.1046/j.1462-5822.2003.00302.x.

Melander, A. L. 1914. "Can Insects become resistant to sprays?" *Journal of Economic Entomology* 7 (2): 167. doi: 10.1093/jee/7.2.167.

Merchant, A., G. Joseph, Q. Wang, S. Brennan, and W. Matsui. 2010. "Gli1 regulates the proliferation and differentiation of HSCs and myeloid progenitors." *Blood* 115 (12): 2391–96. doi: 10.1182/blood-2009-09-241703.

Merino, M. M., C. Rhiner, J. M. Lopez-Gay, D. Buechel, B. Hauert, and E. Moreno. 2015. "Elimination of unfit cells maintains tissue health and prolongs lifespan." *Cell* 160 (3): 461–76. doi: 10.1016/j.cell.2014.12.017.

Merz, B. 1989. "Walking, jumping, collaboration, and competition characterize race to cystic fibrosis gene." *JAMA* 262 (12): 1573–74. doi: 10.1001/jama.1989.03430120023006.

Milan, N. F., B. Z. Kacsoh, and T. A. Schlenke. 2012. "Alcohol consumption as self-medication against blood-borne parasites in the fruit fly." *Curr Biol* 22 (6): 488–93. doi: 10.1016/j.cub.2012.01.045.

Miller, C. 2000. "An overview of the potassium channel family." *Genome Biol* 1 (4): reviews0004. doi: 10.1186/gb-2000-1-4-reviews0004.

Mirth, C. K., and A. W. Shingleton. 2012. "Integrating body and organ size in *Drosophila*: recent advances and outstanding problems." *Front Endocrinol (Lausanne)* 3:49. doi: 10.3389/fendo.2012.00049.

Mitchell, C. L., R. D. Yeager, Z. J. Johnson, S. E. D'Annunzio, K. R. Vogel, and T. Werner. 2015. "Long-term resistance of *Drosophila melanogaster* to the mushroom toxin alpha-amanitin." *PLoS One* 10 (5): e0127569. doi: 10.1371/journal.pone.0127569.

Moberg, K. H., D. W. Bell, D. C. Wahrer, D. A. Haber, and I. K. Hariharan. 2001. "Archipelago regulates Cyclin E levels in *Drosophila* and is mutated in human cancer cell lines." *Nature* 413 (6853): 311–16. doi: 10.1038/35095068.

modENCODE Consortium, S. Roy, J. Ernst, P. V. Kharchenko, P. Kheradpour, N. Negre, M. L. Eaton, et al. 2010. "Identification of functional elements and regulatory circuits by *Drosophila* modENCODE." *Science* 330 (6012): 1787–97. doi: 10.1126/science.1198374.

Mohammad, F., S. Aryal, J. Ho, J. C. Stewart, N. A. Norman, T. L. Tan, A. Eisaka, and A. Claridge-Chang. 2016. "Ancient anxiety pathways influence *Drosophila* defense behaviors." *Curr Biol* 26 (7): 981–86. doi: 10.1016/j.cub.2016.02.031.

Montcouquiol, M., R. A. Rachel, P. J. Lanford, N. G. Copeland, N. A. Jenkins, and M. W. Kelley. 2003. "Identification of *Vangl2* and *Scrb1* as planar polarity genes in mammals." *Nature* 423 (6936): 173–77. doi: 10.1038/nature01618.

Montell, C. and G. M. Rubin. 1989. "Molecular characterization of the *Drosophila trp* locus: a putative integral membrane protein required for phototransduction." *Neuron* 2(4): 1313–23. doi: 10.1016/0896-6273(89)90069-X.

Montell, C. 2009. "A taste of the *Drosophila* gustatory receptors." *Curr Opin Neurobiol* 19 (4): 345–53. doi: 10.1016/j.conb.2009.07.001.

Montell, C. 2011. "The history of TRP channels, a commentary and reflection." *Pflugers Arch* 461 (5): 499–506. doi: 10.1007/s00424-010-0920-3.

Montell, C. 2012. "*Drosophila* visual transduction." *Trends Neurosci* 35 (6): 356–63. doi: 10.1016/j.tins.2012.03.004.

Moore, M. S., J. DeZazzo, A. Y. Luk, T. Tully, C. M. Singh, and U. Heberlein. 1998. "Ethanol intoxication in *Drosophila*: genetic and pharmacological evidence for regulation by the cAMP signaling pathway." *Cell* 93 (6): 997–1007. doi: 10.1016/S0092-8674(00)81205-2.

Moraes, F., and A. Goes. 2016. "A decade of human genome project conclusion: scientific diffusion about our genome knowledge." *Biochem Mol Biol Educ* 44 (3): 215–23. doi: 10.1002/bmb.20952.

Morata, G., and P. Ripoll. 1975. "Minutes: mutants of *Drosophila* autonomously affecting cell division rate." *Dev Biol* 42 (2): 211–21. doi: 10.1016/0012-1606(75)90330-9.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha,. 2009. "A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*." *Cell* 139 (7): 1268–78. doi: 10.1016/j.cell.2009.11.042.

Moreno, E., K. Basler, and G. Morata. 2002. "Cells compete for Decapentaplegic survival factor to prevent apoptosis in *Drosophila* wing development." *Nature* 416 (6882): 755–59. doi: 10.1038/416755a.

Morgan, T. H. 1910. "Sex limited inheritance in *Drosophila*." *Science* 32 (812): 120–22. doi: 10.1126/science.32.812.120.

Morgan, T. H. 1915. "The infertility of rudimentary winged females of *Drosophila ampelophila*." *American Naturalist* 49 (580): 240–50. http://www.jstor.org/stable/2455970.

Morgan, T. H. 1964. *The theory of the gene: Yale University Mrs. Hepsa Ely Silliman memorial lectures*. New York: Hafner.

Moroishi, T., C. G. Hansen, and K. L. Guan. 2015. "The emerging roles of YAP and TAZ in cancer." *Nat Rev Cancer* 15 (2): 73–79. doi: 10.1038/nrc3876.

Morton, R. A. 1993. "Evolution of *Drosophila* insecticide resistance." *Genome* 36 (1): 1–7. doi: 10.1139/g93-001.

Mozgova, I., and L. Hennig. 2015. "The Polycomb group protein regulatory network." *Annu Rev Plant Biol* 66:269–96. doi: 10.1146/annurev-arplant-043014-115627.

Mueller, L. D., P. Shahrestani, C. L. Rauser, and M. R. Rose. 2016. "The death spiral: predicting death in *Drosophila* cohorts." *Biogerontology* 17 (5–6): 805–16. doi: 10.1007/s10522-016-9639-7.

Muller, H. J. 1925. "The regionally differential effect of X rays on crossing over in autosomes of *Drosophila*." *Genetics* 10 (5): 470–507. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1200874/.

Muller, H. J. 1927. "Artificial transmutation of the gene." *Science* 66 (1699): 84–87. doi: 10.1126/science.66.1699.84.

Muller, H. J. 1928. "The measurement of gene mutation rate in *Drosophila*, its high variability, and its dependence upon temperature." *Genetics* 13 (4): 279–357. http://www.genetics.org/content/13/4/279.

Muller, H. J. 1949. "Progress and prospects in human genetics." *Am J Hum Genet* 1 (1): 1–18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1716285/.

Muller, P., D. Kuttenkeuler, V. Gesellchen, M. P. Zeidler, and M. Boutros. 2005. "Identification of JAK/STAT signalling components by genome-wide RNA interference." *Nature* 436 (7052): 871–75. doi: 10.1038/nature03869.

Munoz-Soriano, V., Y. Belacortu, and N. Paricio. 2012. "Planar cell polarity signaling in collective cell movements during morphogenesis and disease." *Curr Genomics* 13 (8): 609–22. doi: 10.2174/138920212803759721.

Murthy, M. 2010. "Unraveling the auditory system of *Drosophila*." *Curr Opin Neurobiol* 20 (3): 281–87. doi: 10.1016/j.conb.2010.02.016.

Navrotskaya, V., G. Oxenkrug, L. Vorobyova, and P. Summergrad. 2016. "Attenuation of high sucrose diet-induced insulin resistance in ABC transporter deficient *white* mutant of *Drosophila melanogaster*." *Integr Obes Diabetes* 2 (2): 187–90. 10.15761/IOD.1000142.

Neckameyer, W. S., and P. Bhatt. 2016. "Protocols to study behavior in *Drosophila*." *Methods Mol Biol* 1478:303–20. doi: 10.1007/978-1-4939-6371-3-19.

Neher, E., B. Sakmann, and J. H. Steinbach. 1978. "The extracellular patch clamp: a method for resolving currents through individual open channels in biological membranes." *Pflügers Arch* 375 (2): 219–28. doi: 10.1007/BF00584247.

Neumüller, R. A., C. Richter, A. Fischer, M. Novatchkova, K. G. Neumüller, and J. A. Knoblich. 2011. "Genome-wide analysis of self-renewal in *Drosophila* neural stem cells by transgenic RNAi." *Cell Stem Cell* 8 (5): 580–93. doi: 10.1016/j.stem.2011.02.022.

Nilius, B., T. Voets, and J. Peters. 2005. "TRP channels in disease." *Sci STKE* 2005 (295): re8. doi: 10.1126/stke.2952005re8.

Nix, N. M., O. Burdine, and M. Walker. 2014. "Vismodegib: first-in-class Hedgehog pathway inhibitor for metastatic or locally advanced basal cell carcinoma." *J Adv Pract Oncol* 5 (4): 294–96. doi: 10.6004/jadpro.2014.5.4.7.

Nobel Media. 2016. "The Nobel Prize in Physiology or Medicine 1933." Accessed September 7. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1933/.

Nobel Media. 2017. "The Nobel Prize in Physiology or Medicine 2017." Accessed October 2. https://www.nobelprize.org/nobel_prizes/medicine/laureates/2017/.

Noree, C., B. K. Sato, R. M. Broyer, and J. E. Wilhelm. 2010. "Identification of novel filament-forming proteins in *Saccharomyces cerevisiae* and *Drosophila melanogaster*." *J Cell Biol* 190 (4): 541–51. doi: 10.1083/jcb.201003001.

Ntziachristos, P., J. S. Lim, J. Sage, and I. Aifantis. 2014. "From fly wings to targeted cancer therapies: a centennial for Notch signaling." *Cancer Cell* 25 (3): 318–34. doi: 10.1016/j.ccr.2014.02.018.

Nussbaum, R. L. 1998. "Putting the Parkin into Parkinson's." *Nature* 392 (6676): 544–45. doi: 10.1038/33271.

Nüsslein-Volhard, C. 1977. "Genetic analysis of pattern-formation in the embryo of *Drosophila melanogaster*: characterization of the maternal-effect mutant *Bicaudal*." *Wilehm Roux Arch Dev Biol* 183 (3): 249–268. doi: 10.1007/BF00867325.

Nüsslein-Volhard, C., and E. Wieschaus. 1980. "Mutations affecting segment number and polarity in *Drosophila*." *Nature* 287 (5785): 795–801. doi: 10.1038/287795a0.

Nutt, J. G., and G. F. Wooten. 2005. "Clinical practice: diagnosis and initial management of Parkinson's disease." *N Engl J Med* 353 (10): 1021–27. doi: 10.1056/NEJMcp043908.

Nybakken, K., S. A. Vokes, T. Y. Lin, A. P. McMahon, and N. Perrimon. 2005. "A genome-wide RNA interference screen in *Drosophila melanogaster* cells for new components of the Hh signaling pathway." *Nat Genet* 37 (12): 1323–32. doi: 10.1038/ng1682.

Oba, Y., M. Ojika, and S. Inouye. 2004. "Characterization of *CG6178* gene product with high sequence similarity to firefly luciferase in *Drosophila melanogaster*." *Gene* 329:137–45. doi: 10.1016/j.gene.2003.12.026.

O'Brien, L. E., and D. Bilder. 2013. "Beyond the niche: tissue-level coordination of stem cell dynamics." *Annu Rev Cell Dev Biol* 29:107–36. doi: 10.1146/annurev-cellbio-101512-122319.

Oertel, W., and J. B. Schulz. 2016. "Current and experimental treatments of Parkinson disease: A guide for neuroscientists." *J Neurochem* 139 Suppl 1:325–37. doi: 10.1111/jnc.13750.

Ojelade, S. A., T. Jia, A. R. Rodan, T. Chenyang, J. L. Kadrmas, A. Cattrell, B. Ruggeri, et al. 2015. "Rsu1 regulates ethanol consumption in *Drosophila* and humans." *Proc Natl Acad Sci U S A* 112 (30): E4085–93. doi: 10.1073/pnas.1417222112.

Okamoto, N., N. Yamanaka, Y. Yagi, Y. Nishida, H. Kataoka, M. B. O'Connor, and A. Mizoguchi. 2009. "A fat body-derived IGF-like peptide regulates post-

feeding growth in *Drosophila*." *Dev Cell* 17 (6): 885–91. doi: 10.1016/j.devcel.2009.10.008.

Oldroyd, H. 1965. *The natural history of flies*. World Naturalist. New York: Norton.

Organisti, C., I. Hein, I. C. Grunwald Kadow, and T. Suzuki. 2015. "Flamingo, a seven-pass transmembrane cadherin, cooperates with Netrin/Frazzled in *Drosophila* midline guidance." *Genes Cells* 20 (1): 50–67. doi: 10.1111/gtc.12202.

Owald, D., S. Lin, and S. Waddell. 2015. "Light, heat, action: neural control of fruit fly behaviour." *Philos Trans R Soc Lond B Biol Sci* 370 (1677): 20140211. doi: 10.1098/rstb.2014.0211.

Paabo, S. 2015. "The diverse origins of the human gene pool." *Nat Rev Genet* 16 (6): 313–14. doi: 10.1038/nrg3954.

Pagliarini, R. A., and T. Xu. 2003. "A genetic screen in *Drosophila* for metastatic behavior." *Science* 302 (5648): 1227–31. doi: 10.1126/science.1088474.

Panayidou, S., E. Ioannidou, and Y. Apidianakis. 2014. "Human pathogenic bacteria, fungi, and viruses in *Drosophila*: disease modeling, lessons, and shortcomings." *Virulence* 5 (2): 253–69. doi: 10.4161/viru.27524.

Pandey, U. B., and C. D. Nichols. 2011. "Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery." *Pharmacol Rev* 63 (2): 411–36. doi: 10.1124/pr.110.003293.

Park, I. H., R. Zhao, J. A. West, A. Yabuuchi, H. Huo, T. A. Ince, P. H. Lerou, M. W. Lensch, and G. Q. Daley. 2008. "Reprogramming of human somatic cells to pluripotency with defined factors." *Nature* 451 (7175): 141–46. doi: 10.1038/nature06534.

Park, J., G. Lee, and J. Chung. 2009. "The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process." *Biochem Biophys Res Commun* 378 (3): 518–23. doi: 10.1016/j.bbrc.2008.11.086.

Park, J., S. B. Lee, S. Lee, Y. Kim, S. Song, S. Kim, E. Bae, et al. 2006. "Mitochondrial dysfunction in Drosophila *PINK1* mutants is complemented by *parkin*." *Nature* 441 (7097): 1157–61. doi: 10.1038/nature04788.

Parkinson, J. 2002. "An essay on the shaking palsy. 1817." *J Neuropsychiatry Clin Neurosci* 14 (2): 223–36; discussion 222. doi: 10.1176/jnp.14.2.223.

Patterson, R. A., M. T. Juarez, A. Hermann, R. Sasik, G. Hardiman, and W. McGinnis. 2013. "Serine proteolytic pathway activation reveals an expanded ensemble of wound response genes in *Drosophila*." *PLoS One* 8 (4): e61773. doi: 10.1371/journal.pone.0061773.

Payne, F. 1910. "Forty-nine generations in the dark." *Biological Bulletin* 18 (4): 188–90. http://www.journals.uchicago.edu/doi/10.2307/1536013.

Peairs, L. M., and D. Sanderson, 1941. *Insect Pests of Farm, Garden, and Orchard*. 4th ed. New York, London: J. Wiley & Sons; Chapman & Hall, Limited.

Pearl, R. 1922. *The biology of death: being a series of lectures delivered at the Lowell Institute in Boston in December 1920*. Monographs on Experimental Biology. Philadelphia; London: J.B. Lippincott.

Pearl, R. 1924a. "Alcohol and Life Duration." *Br Med J* 1 (3309): 948–50.

Pearl, R. 1924b. "The Influence of Alcohol on Duration of Life." *Proc Natl Acad Sci U S A* 10 (6): 231–37. http://www.jstor.org/stable/85135.

Pearl, R. 1928. *The rate of living, being an account of some experimental studies on the biology of life duration.* New York: Knopf.

Peek, M. Y., and G. M. Card. 2016. "Comparative approaches to escape." *Curr Opin Neurobiol* 41:167–73. doi: 10.1016/j.conb.2016.09.012.

Peltan, A., L. Briggs, G. Matthews, S. T. Sweeney, and D. F. Smith. 2012. "Identification of *Drosophila* gene products required for phagocytosis of *Leishmania donovani*." *PLoS One* 7 (12): e51831. doi: 10.1371/journal.pone.0051831.

Pendse, J., P. V. Ramachandran, J. Na, N. Narisu, J. L. Fink, R. L. Cagan, F. S. Collins, and T. J. Baranski. 2013. "A *Drosophila* functional evaluation of candidates from human genome-wide association studies of type 2 diabetes and related metabolic traits identifies tissue-specific roles for *dHHEX*." *BMC Genomics* 14:136. doi: 10.1186/1471-2164-14-136.

Penzo-Méndez, A. I., Y. J. Chen, J. Li, E. S. Witze, and B. Z. Stanger. 2015. "Spontaneous cell competition in immortalized mammalian cell lines." *PLoS One* 10 (7): e0132437. doi: 10.1371/journal.pone.0132437.

Penzo-Méndez, A. I., and B. Z. Stanger. 2014. "Cell competition in vertebrate organ size regulation." *Wiley Interdiscip Rev Dev Biol* 3 (6): 419–27. doi: 10.1002/wdev.148.

Perkins, J. H. 1977. "Edward Fred Knipling's sterile-male technique for control of the screwworm fly." *Environmental Review* 2 (5): 19–37. doi: 10.2307/3984405.

Perkins, L. A., I. Larsen, and N. Perrimon. 1992. "*corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso." *Cell* 70 (2): 225–36. doi: 10.1016/0092-8674(92)90098-W.

Perkins, L. A., M. R. Johnson, M. B. Melnick, and N. Perrimon. 1996. "The nonreceptor protein tyrosine phosphatase Corkscrew functions in multiple receptor tyrosine kinase pathways in Drosophila." *Dev Biol* 180 (1): 63–81. doi: 10.1006/dbio.1996.0285.

Perrimon, N., L. Engstrom, and A. P. Mahowald. 1984. "The effects of zygotic lethal mutations on female germ-line functions in *Drosophila*." *Dev Biol* 105 (2): 404–14. doi: 10.1016/0012-1606(84)90297-5.

Perrimon, N., L. Engstrom, and A. P. Mahowald. 1989. "Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the X chromosome." *Genetics* 121 (2): 333–52. http://www.genetics.org/content/121/2/333.

Perrimon, N., A. Lanjuin, C. Arnold, and E. Noll. 1996. "Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by P-element-induced mutations." *Genetics* 144 (4): 1681–92. http://www.genetics.org/content/144/4/1681.

Perry, T., P. Batterham, and P. J. Daborn. 2011. "The biology of insecticidal activity and resistance." *Insect Biochem Mol Biol* 41 (7): 411–22. http://doi.org/10.1016/j.ibmb.2011.03.003.

Pfeiffer, K., and U. Homberg. 2014. "Organization and functional roles of the central complex in the insect brain." *Annu Rev Entomol* 59:165–84. doi: 10.1146/annurev-ento-011613-162031.

Pfleger, C. M. 2017. "The Hippo Pathway: a master regulatory network important in development and dysregulated in disease." *Curr Top Dev Biol* 123:181–228. doi: 10.1016/bs.ctdb.2016.12.001.

Phillips, J. P., J. Willms, and A. Pitt. 1982. "Alpha-amanitin resistance in three wild strains of *Drosophila melanogaster*." *Can J Genet Cytol* 24 (2): 151–62. doi: 10.1139/g82-014.

Pierre, S., A. S. Bats, and X. Coumoul. 2011. "Understanding SOS (Son of Sevenless)." *Biochem Pharmacol* 82 (9): 1049–56. doi: 10.1016/j.bcp. 2011.07.072.

Pigeault, R., R. Garnier, A. Rivero, and S. Gandon. 2016. "Evolution of transgenerational immunity in invertebrates." *Proc Biol Sci* 283 (1839). doi: 10.1098/rspb.2016.1136.

Podratz, J. L., N. P. Staff, J. B. Boesche, N. J. Giorno, M. E. Hainy, S. A. Herring, M. T. Klennert, et al. 2013. "An automated climbing apparatus to measure chemotherapy-induced neurotoxicity in *Drosophila melanogaster*." *Fly (Austin)* 7 (3): 187–92. doi: 10.4161/fly.24789.

Poodry, C. A., and L. Edgar. 1979. "Reversible alteration in the neuromuscular junctions of *Drosophila melanogaster* bearing a temperature-sensitive mutation, shibire." *J Cell Biol* 81 (3): 520–27. doi: 10.1083/jcb.81.3.520.

Poodry, C. A., L. Hall, and D. T. Suzuki. 1973. "Developmental properties of *shibire*^{ts1}: a pleiotropic mutation affecting larval and adult locomotion and development." *Dev Biol* 32 (2): 373–86. doi: 10.1016/0012-1606(73)90248-0.

Port, F., and S. L. Bullock. 2016. "Augmenting CRISPR applications in *Drosophila* with tRNA-flanked sgRNAs." *Nat Methods* 13 (10): 852–54. doi: 10.1038/nmeth.3972.

Port, F., H. M. Chen, T. Lee, and S. L. Bullock. 2014. "Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*." *Proc Natl Acad Sci U S A* 111 (29): E2967–76. doi: 10.1073/pnas.1405500111.

Porteous, D. J., and J. R. Dorin. 1991. "Cystic fibrosis. 3. Cloning the cystic fibrosis gene: implications for diagnosis and treatment." *Thorax* 46 (1): 46–55. doi: 10.1136/thx.46.1.46.

Pospisilik, J. A., D. Schramek, H. Schnidar, S. J. Cronin, N. T. Nehme, X. Zhang, C. Knauf, et al. 2010. "*Drosophila* genome-wide obesity screen reveals Hedgehog as a determinant of brown versus white adipose cell fate." *Cell* 140 (1): 148–60. doi: 10.1016/j.cell.2009.12.027.

Psychiatric Genomics Consortium. 2017. "Psychiatric Genomics Consortium." Accessed September 24. http://www.med.unc.edu/pgc.

Puro, J., and T. Nygren. 1975. "Mode of action of a homoeotic gene in *Drosophila melanogaster*: localization and dosage effects of *Polycomb*." *Hereditas* 81 (2): 237–48. doi: 10.1111/j.1601-5223.1975.tb01038.x.

Quinonez, S. C., and J. W. Innis. 2014. "Human HOX gene disorders." *Mol Genet Metab* 111 (1): 4–15. doi: 10.1016/j.ymgme.2013.10.012.

Quiring, R., U. Walldorf, U. Kloter, and W. J. Gehring. 1994. "Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans." *Science* 265 (5173): 785–89. http://www.jstor.org/stable/2884323.

Rajan, A., and N. Perrimon. 2012. "*Drosophila* cytokine Unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion." *Cell* 151 (1): 123–37. doi: 10.1016/j.cell.2012.08.019.

Ramoni, R. B., J. J. Mulvihill, D. R. Adams, P. Allard, E. A. Ashley, J. A. Bernstein, W. A. Gahl, et al. 2017. "The Undiagnosed Diseases Network: accelerating discovery about health and disease." *Am J Hum Genet* 100 (2): 185–92. doi: 10.1016/j.ajhg.2017.01.006.

Rappuoli, R., C. W. Mandl, S. Black, and E. De Gregorio. 2011. "Vaccines for the twenty-first century society." *Nat Rev Immunol* 11 (12): 865–72. doi: 10.1038/nri3085.

Rera, M., S. Bahadorani, J. Cho, C. L. Koehler, M. Ulgherait, J. H. Hur, W. S. Ansari, T. Lo, Jr., D. L. Jones, and D. W. Walker. 2011. "Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog." *Cell Metab* 14 (5): 623–34. doi: 10.1016/j.cmet.2011.09.013.

Rera, M., R. I. Clark, and D. W. Walker. 2012. "Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*." *Proc Natl Acad Sci U S A* 109 (52): 21528–33. doi: 10.1073/pnas.1215849110.

Rideout, E. J., M. S. Narsaiya, and S. S. Grewal. 2015. "The sex determination gene *transformer* regulates male-female differences in *Drosophila* body size." *PLoS Genet* 11 (12): e1005683. doi: 10.1371/journal.pgen.1005683.

Rincon-Limas, D. E., K. Jensen, and P. Fernandez-Funez. 2012. "*Drosophila* models of proteinopathies: the little fly that could." *Curr Pharm Des.* 18 (8): 1108–22. doi: 10.2174/138161212799315894.

Ritossa, F. 1996. "Discovery of the heat shock response." *Cell Stress Chaperones* 1 (2): 97–98. http://www.jstor.org/stable/1601907.

Robinson, A. S. 2002. "Mutations and their use in insect control." *Mutat Res* 511 (2): 113–32. doi: 10.1016/S1383-5742(02)00006-6.

Robinson, S. W., P. Herzyk, J. A. Dow, and D. P. Leader. 2013. "FlyAtlas: database of gene expression in the tissues of *Drosophila melanogaster*." *Nucleic Acids Res* 41 (Database issue): D744–50. doi: 10.1093/nar/gks1141.

Roeder, T., K. Isermann, K. Kallsen, K. Uliczka, and C. Wagner. 2012. "A *Drosophila* asthma model—what the fly tells us about inflammatory diseases of the lung." *Adv Exp Med Biol* 710:37–47. doi: 10.1007/978-1-4419-5638-5_5.

Rogers, J., and R. A. Gibbs. 2014. "Comparative primate genomics: emerging patterns of genome content and dynamics." *Nat Rev Genet* 15 (5): 347–59. doi: 10.1038/nrg3707.

Rommens, J. M., M. C. Iannuzzi, B. Kerem, M. L. Drumm, G. Melmer, M. Dean, R. Rozmahel, et al. 1989. "Identification of the cystic fibrosis gene: chromosome walking and jumping." *Science* 245 (4922): 1059–65. doi: 10.1016/0168-9525(89)90154-6.

Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm, G. M. Rubin, K. Weigmann, et al. 1998. "Systematic gain-of-function genetics in Drosophila." *Development* 125 (6): 1049–57. http://dev.biologists.org/content/125/6/1049.

Rubin, G. M. 2015. "FlyBook: A Preface." *Genetics* 201 (2): 343. doi: 10.1534/genetics.115.182220.

Rubin, G. M., and A. C. Spradling. 1982. "Genetic transformation of *Drosophila* with transposable element vectors." *Science* 218 (4570): 348–53. doi: 10.1126/science.6289436.

Rui, Y. N., Z. Xu, B. Patel, Z. Chen, D. Chen, A. Tito, G. David, et al. 2015. "Huntingtin functions as a scaffold for selective macroautophagy." *Nat Cell Biol* 17 (3): 262–75. doi: 10.1038/ncb3101.

Rulifson, E. J., S. K. Kim, and R. Nusse. 2002. "Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes." *Science* 296 (5570): 1118–20. doi: 10.1126/science.1070058.

Ruppert, J. M., K. W. Kinzler, A. J. Wong, S. H. Bigner, F. T. Kao, M. L. Law, H. N. Seuanez, S. J. O'Brien, and B. Vogelstein. 1988. "The *GLI*-Kruppel family of human genes." *Mol Cell Biol* 8 (8): 3104–13. doi: 10.1128/MCB.8.8.3104.

Ruppert, J. M., B. Vogelstein, K. Arheden, and K. W. Kinzler. 1990. "*GLI3* encodes a 190-kilodalton protein with multiple regions of GLI similarity." *Mol Cell Biol* 10 (10): 5408–15. doi: 10.1128/MCB.10.10.5408.

Sadanandappa, M. K., and M. Ramaswami. 2013. "Fly model causes neurological rethink." *Elife* 2:e01820. doi: 10.7554/eLife.01820.

Saucedo, L. J., and B. A. Edgar. 2007. "Filling out the Hippo pathway." *Nat Rev Mol Cell Biol* 8 (8): 613–21. doi: 10.1038/nrm2221.

Savic, N., and G. Schwank. 2016. "Advances in therapeutic CRISPR/Cas9 genome editing." *Transl Res* 168:15–21. doi: 10.1016/j.trsl.2015.09.008.

Schmiegelow, K., H. Eiberg, L. C. Tsui, M. Buchwald, P. D. Phelan, R. Williamson, W. Warwick, E. Niebuhr, J. Mohr, M. Schwartz, and C. Koch. 1986. "Linkage between the loci for cystic fibrosis and paraoxonase." *Clin Genet* 29 (5): 374–77. doi: 10.1111/j.1399-0004.1986.tb00507.x.

Schneider, D., and M. Shahabuddin. 2000. "Malaria parasite development in a *Drosophila* model." *Science* 288 (5475): 2376–79. doi: 10.1126/science.288.5475.2376.

Schofield, R. 1978. "The relationship between the spleen colony-forming cell and the haemopoietic stem cell." *Blood Cells* 4 (1–2): 7–25.

Schubiger, G. 1971. "Regeneration, duplication and transdetermination in fragments of the leg disc of *Drosophila melanogaster*." *Dev Biol* 26 (2): 277–95. http://doi.org/10.1016/0012-1606(71)90127-8.

Schumann, G., L. J. Coin, A. Lourdusamy, P. Charoen, K. H. Berger, D. Stacey, S. Desrivieres, et al. 2011. "Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption." *Proc Natl Acad Sci U S A* 108 (17): 7119–24. doi: 10.1073/pnas.1017288108.

Schüpbach, T., and E. Wieschaus. 1989. "Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations." *Genetics* 121 (1): 101–17. http://www.genetics.org/content/121/1/101.

Schüpbach, T., and E. Wieschaus. 1991. "Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology." *Genetics* 129 (4): 1119–36. http://www.genetics.org/content/129/4/1119.

Scott, M. P., and A. J. Weiner. 1984. "Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of *Drosophila*." *Proc Natl Acad Sci U S A* 81 (13): 4115–19.

Seecof, R. 1968. "The sigma virus infection of *Drosophila melanogaster*." *Curr Top Microbiol Immunol* 42:59–93. 10.1007/978-3-642-46115-6_4.

Seligman, M. E., and S. F. Maier. 1967. "Failure to escape traumatic shock." *J Exp Psychol* 74 (1): 1–9. doi: 10.1037/h0024514.

Senthilan, P. R., D. Piepenbrock, G. Ovezmyradov, B. Nadrowski, S. Bechstedt, S. Pauls, M. Winkler, W. Mobius, J. Howard, and M. C. Gopfert. 2012. "*Drosophila* auditory organ genes and genetic hearing defects." *Cell* 150 (5): 1042–54. doi: 10.1016/j.cell.2012.06.043.

Senti, K. A., and J. Brennecke. 2010. "The piRNA pathway: a fly's perspective on the guardian of the genome." *Trends Genet* 26 (12): 499–509. doi: 10.1016/j.tig.2010.08.007.

Service, M. W. 2012. *Medical entomology for students.* 5th ed. Cambridge: Cambridge University Press.

Shih, C. T., O. Sporns, S. L. Yuan, T. S. Su, Y. J. Lin, C. C. Chuang, T. Y. Wang, C. C. Lo, R. J. Greenspan, and A. S. Chiang. 2015. "Connectomics-based analysis of information flow in the *Drosophila* brain." *Curr Biol* 25 (10): 1249–58. doi: 10.1016/j.cub.2015.03.021.

Shilo, B. Z. 2016. "New twists in Drosophila cell signaling." *J Biol Chem* 291 (15): 7805–8. doi: 10.1074/jbc.R115.711473.

Shulman, J. M. 2015. "*Drosophila* and experimental neurology in the post-genomic era." *Exp Neurol* 274 (Pt A): 4–13. doi: 10.1016/j.expneurol.2015.03.016.

Silies, M., D. M. Gohl, and T. R. Clandinin. 2014. "Motion-detecting circuits in flies: coming into view." *Annu Rev Neurosci* 37:307–27. doi: 10.1146/annurev-neuro-071013-013931.

Simon, J. C., and M. H. Dickinson. 2010. "A new chamber for studying the behavior of *Drosophila*." *PLoS One* 5 (1): e8793. doi: 10.1371/journal.pone.0008793.

Simon, M. A., D. D. Bowtell, G. S. Dodson, T. R. Laverty, and G. M. Rubin. 1991. "Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase." *Cell* 67 (4): 701–16. doi: 10.1016/0092-8674(91)90065-7.

Simon, M. A., T. B. Kornberg, and J. M. Bishop. 1983. "Three loci related to the src oncogene and tyrosine-specific protein kinase activity in *Drosophila*." *Nature* 302 (5911): 837–39. doi: 10.1038/302837a0.

Simons, M., and M. Mlodzik. 2008. "Planar cell polarity signaling: from fly development to human disease." *Annu Rev Genet* 42:517–40. doi: 10.1146/annurev.genet.42.110807.091432.

Simpson, P. 1979. "Parameters of cell competition in the compartments of the wing disc of *Drosophila*." *Dev Biol* 69 (1): 182–93. doi: 10.1016/0012-1606(79)90284-7.

Simpson, P., and G. Morata. 1981. "Differential mitotic rates and patterns of growth in compartments in the *Drosophila* wing." *Dev Biol* 85 (2): 299–308. http://doi.org/10.1016/0012-1606(81)90261-X.

Singh, R. S., and R. A. Morton. 1981. "Selection for malathion-resistance in *Drosophila melanogaster*." *Can J Genet Cytol* 23 (2): 355–69. doi: 10.1139/g81-038.

Singh, S. R., X. Zeng, Z. Zheng, and S. X. Hou. 2011. "The adult *Drosophila* gastric and stomach organs are maintained by a multipotent stem cell pool at the foregut/midgut junction in the cardia (proventriculus)." *Cell Cycle* 10 (7): 1109–20. doi: 10.4161/cc.10.7.14830.

Slack, C., N. Alic, A. Foley, M. Cabecinha, M. P. Hoddinott, and L. Partridge. 2015. "The Ras-Erk-ETS-signaling pathway is a drug target for longevity." *Cell* 162 (1): 72–83. doi: 10.1016/j.cell.2015.06.023.

Slaidina, M., R. Delanoue, S. Gronke, L. Partridge, and P. Leopold. 2009. "A *Drosophila* insulin-like peptide promotes growth during nonfeeding states." *Dev Cell* 17 (6): 874–84. doi: 10.1016/j.devcel.2009.10.009.

Soares, L., M. Parisi, and N. M. Bonini. 2014. "Axon injury and regeneration in the adult *Drosophila*." *Sci Rep* 4:6199. doi: 10.1038/srep06199.

Somer, R. A., and C. S. Thummel. 2014. "Epigenetic inheritance of metabolic state." *Curr Opin Genet Dev* 27:43–47. doi: 10.1016/j.gde.2014.03.008.

Soscia, S. J., J. E. Kirby, K. J. Washicosky, S. M. Tucker, M. Ingelsson, B. Hyman, M. A. Burton, et al. 2010. "The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide." *PLoS One* 5 (3): e9505. doi: 10.1371/journal.pone.0009505.

Speliotes, E. K., C. J. Willer, S. I. Berndt, K. L. Monda, G. Thorleifsson, A. U. Jackson, H. Lango Allen, et al. 2010. "Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index." *Nat Genet* 42 (11): 937–48. doi: 10.1038/ng.686.

Spielmann, M., F. Brancati, P. M. Krawitz, P. N. Robinson, D. M. Ibrahim, M. Franke, J. Hecht, et al. 2012. "Homeotic arm-to-leg transformation associated with genomic rearrangements at the *PITX1* locus." *Am J Hum Genet* 91 (4): 629–35. doi: 10.1016/j.ajhg.2012.08.014.

Spierer, P., A. Spierer, W. Bender, and D. S. Hogness. 1983. "Molecular mapping of genetic and chromomeric units in *Drosophila melanogaster*." *J Mol Biol* 168 (1): 35–50. doi: 10.1016/S0022-2836(83)80321-0.

St Johnston, D. 2002. "The art and design of genetic screens: *Drosophila melanogaster*." *Nat Rev Genet* 3 (3): 176–88. doi: 10.1038/nrg751.

Stanton, B. Z., L. F. Peng, N. Maloof, K. Nakai, X. Wang, J. L. Duffner, K. M. Taveras, et al. 2009. "A small molecule that binds Hedgehog and blocks its signaling in human cells." *Nat Chem Biol* 5 (3): 154–56. doi: 10.1038/nchembio.142.

Stern, C. 1954. "Two or Three Bristles." *American Scientist* 42 (2): 212–47. http://www.jstor.org/stable/27826541.

Stickel, S. A., N. P. Gomes, B. Frederick, D. Raben, and T. T. Su. 2015. "Bouvardin is a radiation modulator with a novel mechanism of action." *Radiat Res* 184 (4): 392–403. doi: 10.1667/RR14068.1.

Stocker, R. F. 1994. "The organization of the chemosensory system in *Drosophila melanogaster*: a review." *Cell Tissue Res* 275 (1): 3–26. doi: 10.1007/BF00305372.

Struhl, G., J. Casal, and P. A. Lawrence. 2012. "Dissecting the molecular bridges that mediate the function of Frizzled in planar cell polarity." *Development* 139 (19): 3665–74. doi: 10.1242/dev.083550.

Strutt, D., and S. J. Warrington. 2008. "Planar polarity genes in the *Drosophila* wing regulate the localisation of the FH3-domain protein Multiple Wing Hairs to control the site of hair production." *Development* 135 (18): 3103–11. doi: 10.1242/dev.025205.

Strutt, H., and D. Strutt. 2005. "Long-range coordination of planar polarity in *Drosophila*." *Bioessays* 27 (12): 1218–27. doi: 10.1002/bies.20318.

Sturtevant, A. H. 1913. "The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association." *Journal of Experimental Zoology* 14 (1): 43–59. doi: 10.1002/jez.1400140104.

Sturtevant, A. H. 1965. *A History of Genetics.* New York: Harper & Row.

Sublett, J. E., R. E. Entrekin, A. T. Look, and D. A. Reardon. 1998. "Chromosomal localization of the human smoothened gene (*SMOH*) to 7q32. 3 by fluorescence in situ hybridization and radiation hybrid mapping." *Genomics* 50 (1): 112–14. doi: 10.1006/geno.1998.5227.

Sudmant, P. H., S. Mallick, B. J. Nelson, F. Hormozdiari, N. Krumm, J. Huddleston, B. P. Coe, et al. 2015. "Global diversity, population stratification, and selection of human copy-number variation." *Science* 349 (6253): aab3761. doi: 10.1126/science.aab3761.

Szakmary, A., D. N. Cox, Z. Wang, and H. Lin. 2005. "Regulatory relationship among *piwi*, *pumilio*, and *bag-of-marbles* in *Drosophila* germline stem cell self-renewal and differentiation." *Curr Biol* 15 (2): 171–78. doi: 10.1016/j.cub.2005.01.005.

Takahashi, K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka. 2007. "Induction of pluripotent stem cells from adult human fibroblasts by defined factors." *Cell* 131 (5): 861–72. doi: 10.1016/j.cell.2007.11.019.

Takebe, N., L. Miele, P. J. Harris, W. Jeong, H. Bando, M. Kahn, S. X. Yang, and S. P. Ivy. 2015. "Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update." *Nat Rev Clin Oncol* 12 (8): 445–64. doi: 10.1038/nrclinonc.2015.61.

Tanimura, A., S. Dan, and M. Yoshida. 1998. "Cloning of novel isoforms of the human Gli2 oncogene and their activities to enhance tax-dependent transcription of the human T-cell leukemia virus type 1 genome." *J Virol* 72 (5): 3958–64. http://jvi.asm.org/content/72/5/3958.

Tao, L., J. Zhang, P. Meraner, A. Tovaglieri, X. Wu, R. Gerhard, X. Zhang, et al. 2016. "Frizzled proteins are colonic epithelial receptors for *C. difficile* toxin B." *Nature*. doi: 10.1038/nature19799.

Tao, Y., A. E. Christiansen, and R. A. Schulz. 2007. "Second chromosome genes required for heart development in *Drosophila melanogaster*." *Genesis* 45 (10): 607–17. doi: 10.1002/dvg.20333.

Tapon, N., K. F. Harvey, D. W. Bell, D. C. Wahrer, T. A. Schiripo, D. Haber, and I. K. Hariharan. 2002. "*salvador* promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines." *Cell* 110 (4): 467–78. doi: 10.1016/S0092-8674(02)00824-3.

Tapon, N., N. Ito, B. J. Dickson, J. E. Treisman, and I. K. Hariharan. 2001. "The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation." *Cell* 105 (3): 345–55. doi: 10.1016/S0092-8674(01)00332-4.

Taylor, J., N. Abramova, J. Charlton, and P. N. Adler. 1998. "*Van Gogh*: a new Drosophila tissue polarity gene." *Genetics* 150 (1): 199–210. http://www.genetics.org/content/150/1/199.

Taylor, K., K. Kleinhesselink, M. D. George, R. Morgan, T. Smallwood, A. S. Hammonds, P. M. Fuller, et al. 2014. "Toll mediated infection response is altered by gravity and spaceflight in *Drosophila*." *PLoS One* 9 (1): e86485. doi: 10.1371/journal.pone.0086485.

Teixeira, L., A. Ferreira, and M. Ashburner. 2008. "The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*." *PLoS Biol* 6 (12): e2. doi: 10.1371/journal.pbio.1000002.

Thomas, F., R. Poulin, J. F. Guegan, Y. Michalakis, and F. Renaud. 2000. "Are there pros as well as cons to being parasitized?" *Parasitol Today* 16 (12): 533–36. doi: 10.1016/S0169-4758(00)01790-7.

Thomas, S., K. H. Fisher, J. A. Snowden, S. J. Danson, S. Brown, and M. P. Zeidler. 2015. "Methotrexate is a JAK/STAT Pathway Inhibitor." *PLoS One* 10 (7): e0130078. doi: 10.1371/journal.pone.0130078.

Tidbury, H. J., A. B. Pedersen, and M. Boots. 2011. "Within and transgenerational immune priming in an insect to a DNA virus." *Proc Biol Sci* 278 (1707): 871–76. doi: 10.1098/rspb.2010.1517.

Tokunaga, C. 1966. "New mutants: report of C. Tokunaga." *Drosophila Information Service* 41:57.

Tomczak, K., P. Czerwinska, and M. Wiznerowicz. 2015. "The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge." *Contemp Oncol (Pozn)* 19 (1A): A68–77. doi: 10.5114/wo.2014.47136.

Tracey, W. D., Jr., R. I. Wilson, G. Laurent, and S. Benzer. 2003. "*painless*, a *Drosophila* gene essential for nociception." *Cell* 113 (2): 261–73. doi: 10.1016/S0092-8674(03)00272-1.

Trentin, J. J. 1970. "Influence of hematopoietic organ stroma (hematopoietic inductive microenvironments) on stem cell differentiation." In *Regulation of hematopoiesis,* edited by A. S. Gordon. New York: Appleton-Century-Crofts.

Tsai, S. Q., and J. K. Joung. 2016. "Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases." *Nat Rev Genet* 17 (5): 300–312. doi: 10.1038/nrg.2016.28.

Tulina, N., and E. Matunis. 2001. "Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling." *Science* 294 (5551): 2546–49. doi: 10.1126/science.1066700.

Turelli, M., and N. H. Barton. 2017. "Deploying dengue-suppressing *Wolbachia*: robust models predict slow but effective spatial spread in *Aedes aegypti*." *Theor Popul Biol* 115:45–60. doi: 10.1016/j.tpb.2017.03.003.

Turnbull, J. E. 1999. "Introduction: Heparan sulfate proteoglycans: multifunctional regulators of growth-factor function." In *Cell surface proteoglycans in signalling and development,* edited by A. Lander, Nakato, H., Selleck, S. B., Turnbull, J. E., Coath, C. Strasbourg: Human Frontier Science Program.

Tuschl, T., P. D. Zamore, R. Lehmann, D. P. Bartel, and P. A. Sharp. 1999. "Targeted mRNA degradation by double-stranded RNA in vitro." *Genes Dev* 13 (24): 3191–97. http://genesdev.cshlp.org/content/13/24/3191.

Twilley, N. 2015. "L. A.'s Back-Yard Entomologists." *Elements* (blog), *New Yorker,* July 7. http://www.newyorker.com/tech/elements/los-angeles-back-yard-entomologists.

Undiagnosed Diseases Network. 2017. "Undiagnosed Diseases Network." Accessed June 4. https://undiagnosed.hms.harvard.edu/.

Usui, T., Y. Shima, Y. Shimada, S. Hirano, R. W. Burgess, T. L. Schwarz, M. Takeichi, and T. Uemura. 1999. "Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled." *Cell* 98 (5): 585–95. doi: 10.1016/S0092-8674(00)80046-X.

van Alphen, B., and B. van Swinderen. 2013. "*Drosophila* strategies to study psychiatric disorders." *Brain Res Bull* 92:1–11. doi: 10.1016/j.brainresbull.2011.09.007.

van der Voet, M., B. Nijhof, M. A. Oortveld, and A. Schenck. 2014. "*Drosophila* models of early onset cognitive disorders and their clinical applications." *Neurosci Biobehav Rev* 46 Pt 2:326–42. doi: 10.1016/j.neubiorev.2014.01.013.

van Swinderen, B. 2005. "The remote roots of consciousness in fruit-fly selective attention?" *Bioessays* 27 (3): 321–30. doi: 10.1002/bies.20195.

Vavre, F., L. Mouton, and B. A. Pannebakker. 2009. "*Drosophila*-parasitoid communities as model systems for host-*Wolbachia* interactions." *Adv Parasitol* 70:299–331. doi: 10.1016/S0065-308X(09)70012-0.

Venken, K. J., and H. J. Bellen. 2012. "Genome-wide manipulations of *Drosophila melanogaster* with transposons, Flp recombinase, and PhiC31 integrase." *Methods Mol Biol* 859:203–28. doi: 10.1007/978-1-61779-603-6-12.

Vidal, M., S. Wells, A. Ryan, and R. Cagan. 2005. "ZD6474 suppresses oncogenic RET isoforms in a *Drosophila* model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma." *Cancer Res* 65 (9): 3538–41. doi: 10.1158/0008-5472.CAN-04-4561.

Vidal, O. M., W. Stec, N. Bausek, E. Smythe, and M. P. Zeidler. 2010. "Negative regulation of *Drosophila* JAK-STAT signalling by endocytic trafficking." *J Cell Sci* 123 (Pt 20): 3457–66. doi: 10.1242/jcs.066902.

Vig, M., C. Peinelt, A. Beck, D. L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, and J. P. Kinet. 2006. "CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry." *Science* 312 (5777): 1220–23. doi: 10.1126/science.1127883.

Vinayagam, A., M. M. Kulkarni, R. Sopko, X. Sun, Y. Hu, A. Nand, C. Villalta, et al. 2016. "An integrative analysis of the InR/PI3K/Akt network identifies the dynamic response to insulin signaling." *Cell Rep* 16 (11): 3062–74. doi: 10.1016/j.celrep. 2016.08.029.

Vogel, E. W., U. Graf, H. J. Frei, and M. M. Nivard. 1999. "The results of assays in *Drosophila* as indicators of exposure to carcinogens." *IARC Sci Publ* (146): 427–70.

Vollmayr, B., and P. Gass. 2013. "Learned helplessness: unique features and translational value of a cognitive depression model." *Cell Tissue Res* 354 (1): 171–78. doi: 10.1007/s00441-013-1654-2.

Waldbauer, Gilbert. 2003. *What good are bugs? insects in the web of life*. Cambridge, MA: Harvard University Press.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong, et al. 2011. "The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations." *Nature* 476 (7361): 450–53. doi: 10.1038/nature10355.

Wallingford, J. B. 2012. "Planar cell polarity and the developmental control of cell behavior in vertebrate embryos." *Annu Rev Cell Dev Biol* 28:627–53. doi: 10.1146/annurev-cellbio-092910-154208.

Walter, H. E. 1930. *Genetics: an introduction to the study of heredity.* 3rd ed. New York: Macmillan.

Wang, Q., M. E. Curran, I. Splawski, T. C. Burn, J. M. Millholland, T. J. VanRaay, J. Shen, et al. 1996. "Positional cloning of a novel potassium channel gene: *KVLQT1* mutations cause cardiac arrhythmias." *Nat Genet* 12 (1): 17–23. doi: 10.1038/ng0196-17.

Wangler, M. F., Y. Hu, and J. M. Shulman. 2017. "*Drosophila* and genome-wide association studies: a review and resource for the functional dissection of human complex traits." *Dis Model Mech* 10 (2): 77–88. doi: 10.1242/dmm.027680.

Warmke, J., R. Drysdale, and B. Ganetzky. 1991. "A distinct potassium channel polypeptide encoded by the *Drosophila eag* locus." *Science* 252 (5012): 1560–62. http://www.jstor.org/stable/2876117.

Warrick, J. M., H. L. Paulson, G. L. Gray-Board, Q. T. Bui, K. H. Fischbeck, R. N. Pittman, and N. M. Bonini. 1998. "Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*." *Cell* 93 (6): 939–49. doi: 10.1016/S0092-8674(00)81200-3.

Wawersik, S., and R. L. Maas. 2000. "Vertebrate eye development as modeled in *Drosophila*." *Hum Mol Genet* 9 (6): 917–25. doi: 10.1093/hmg/9.6.917.

Weavers, H., S. Prieto-Sanchez, F. Grawe, A. Garcia-Lopez, R. Artero, M. Wilsch-Brauninger, M. Ruiz-Gomez, H. Skaer, and B. Denholm. 2009. "The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm." *Nature* 457 (7227): 322–26. doi: 10.1038/nature07526.

Weber, K. E. 1988. "An apparatus for measurement of resistance to gas-phase reagents." *Drosophila Information Service* 67:90–92.

Webster, C. L., B. Longdon, S. H. Lewis, and D. J. Obbard. 2016. "Twenty-five new viruses associated with the Drosophilidae (Diptera)." *Evol Bioinform Online* 12 (Suppl 2): 13–25. doi: 10.4137/EBO.S39454.

Webster, C. L., F. M. Waldron, S. Robertson, D. Crowson, G. Ferrari, J. F. Quintana, J. M. Brouqui, et al. 2015. "The discovery, distribution, and evolution of viruses associated with *Drosophila melanogaster*." *PLoS Biol* 13 (7): e1002210. doi: 10.1371/journal.pbio.1002210.

Wedeen, C., K. Harding, and M. Levine. 1986. "Spatial regulation of antennapedia and bithorax gene expression by the *Polycomb* locus in Drosophila." *Cell* 44 (5): 739–48. doi: 10.1016/0092-8674(86)90840-8.

Wehrli, M., and A. Tomlinson. 1995. "Epithelial planar polarity in the developing *Drosophila* eye." *Development* 121 (8): 2451–59. http://dev.biologists.org/content/121/8/2451.

Weiner, J. 1999. *Time, love, memory: a great biologist and his quest for the origins of behavior*. New York: Knopf.

Weismann, A. 1889. *Essays upon heredity and kindred biological problems*. Unspecified translation. Oxford: Clarendon.

Werren, J. H., L. Baldo, and M. E. Clark. 2008. "*Wolbachia*: master manipulators of invertebrate biology." *Nat Rev Microbiol* 6 (10): 741–51. doi: 10.1038/nrmicro1969.

West, L. S. 1951. *The housefly: its natural history, medical importance, and control*. Ithaca, NY: Comstock.

Wieschaus, E., and C. Nüsslein-Volhard. 2014. "Walter Gehring (1939–2014)." *Curr Biol* 24 (14): R632–34. doi: 10.1016/j.cub.2014.06.039.

Wieschaus, E., and C. Nüsslein-Volhard. 2016. "The Heidelberg screen for pattern mutants of *Drosophila*: a personal account." *Annu Rev Cell Dev Biol*. doi: 10.1146/annurev-cellbio-113015-023138.

Wigglesworth, V. B. 1965. *The principles of insect physiology*. 6th ed. London: Methuen.

Williams, M. J., A. Eriksson, M. Shaik, S. Voisin, O. Yamskova, J. Paulsson, K. Thombare, R. Fredriksson, and H. B. Schioth. 2015. "The obesity-linked gene *Nudt3 Drosophila* homolog *Aps* is associated with insulin signaling." *Mol Endocrinol* 29 (9): 1303–19. doi: 10.1210/ME.2015-1077.

Wilson, T. M. 1907. "On the chemistry and staining properties of certain derivatives of the methylene blue group when combined with eosin." *J Exp Med* 9 (6): 645–70. doi: 10.1084/jem.9.6.645.

Wolf, F. W., and U. Heberlein. 2003. "Invertebrate models of drug abuse." *J Neurobiol* 54 (1): 161–78. doi: 10.1002/neu.10166.

Wolff, T., N. A. Iyer, and G. M. Rubin. 2015. "Neuroarchitecture and neuroanatomy of the *Drosophila* central complex: A GAL4-based dissection of protocerebral bridge neurons and circuits." *J Comp Neurol* 523 (7): 997–1037. doi: 10.1002/cne.23705.

Woodard, C., T. Huang, H. Sun, S. L. Helfand, and J. Carlson. 1989. "Genetic analysis of olfactory behavior in *Drosophila*: a new screen yields the *ota* mutants." *Genetics* 123 (2): 315–26. http://www.genetics.org/content/123/2/315.

Wu, M. N., K. Koh, Z. Yue, W. J. Joiner, and A. Sehgal. 2008. "A genetic screen for sleep and circadian mutants reveals mechanisms underlying regulation of sleep in *Drosophila*." *Sleep* 31 (4): 465–72. doi: 10.1093/sleep/31.4.465.

Wu, S., J. Huang, J. Dong, and D. Pan. 2003. "*hippo* encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with *salvador* and *warts*." *Cell* 114 (4): 445–56. doi: 10.1016/S0092-8674(03)00549-X.

Wulff, H., N. A. Castle, and L. A. Pardo. 2009. "Voltage-gated potassium channels as therapeutic targets." *Nat Rev Drug Discov* 8 (12): 982–1001. doi: 10.1038/nrd2983.

Xu, T., W. Wang, S. Zhang, R. A. Stewart, and W. Yu. 1995. "Identifying tumor suppressors in genetic mosaics: the *Drosophila lats* gene encodes a putative

protein kinase." *Development* 121 (4): 1053–63. http://dev.biologists.org/content/121/4/1053.

Yamamoto, S., M. Jaiswal, W. L. Charng, T. Gambin, E. Karaca, G. Mirzaa, W. Wiszniewski, et al. 2014. "A *Drosophila* genetic resource of mutants to study mechanisms underlying human genetic diseases." *Cell* 159 (1): 200–14. doi: 10.1016/j.cell.2014.09.002.

Yan, D., and X. Lin. 2009. "Shaping morphogen gradients by proteoglycans." *Cold Spring Harb Perspect Biol* 1 (3): a002493. doi: 10.1101/cshperspect.a002493.

Yan, D., R. A. Neumüller, M. Buckner, K. Ayers, H. Li, Y. Hu, D. Yang-Zhou, et al. 2014. "A regulatory network of *Drosophila* germline stem cell self-renewal." *Dev Cell* 28 (4): 459–73. doi: 10.1016/j.devcel.2014.01.020.

Yan, J., D. Huen, T. Morely, G. Johnson, D. Gubb, J. Roote, and P. N. Adler. 2008. "The *multiple-wing-hairs* gene encodes a novel GBD-FH3 domain-containing protein that functions both prior to and after wing hair initiation." *Genetics* 180 (1): 219–28. doi: 10.1534/genetics.108.091314.

Yang, J., C. McCart, D. J. Woods, S. Terhzaz, K. G. Greenwood, R. H. ffrench-Constant, and J. A. Dow. 2007. "A *Drosophila* systems approach to xenobiotic metabolism." *Physiol Genomics* 30 (3): 223–31. doi: 10.1152/physiolgenomics.00018.2007.

Yang, Y., S. Gehrke, Y. Imai, Z. Huang, Y. Ouyang, J. W. Wang, L. Yang, M. F. Beal, H. Vogel, and B. Lu. 2006. "Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin." *Proc Natl Acad Sci U S A* 103 (28): 10793–98. doi: 10.1073/pnas.0602493103.

Yang, Y., and M. Mlodzik. 2015. "Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt)." *Annu Rev Cell Dev Biol* 31: 623–46. doi: 10.1146/annurev-cellbio-100814-125315.

Yates, L. L., and C. H. Dean. 2011. "Planar polarity: a new player in both lung development and disease." *Organogenesis* 7 (3): 209–16. doi: 10.4161/org.7.3.18462.

Yayon, A., M. Klagsbrun, J. D. Esko, P. Leder, and D. M. Ornitz. 1991. "Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor." *Cell* 64 (4): 841–48. doi: 10.1016/0092-8674(91)90512-W.

Ye, S., and T. S. Eisinger-Mathason. 2016. "Targeting the Hippo pathway: clinical implications and therapeutics." *Pharmacol Res* 103:270–78. doi: 10.1016/j.phrs.2015.11.025.

Yi, P., Z. Han, X. Li, and E. N. Olson. 2006. "The mevalonate pathway controls heart formation in *Drosophila* by isoprenylation of Ggamma1." *Science* 313 (5791): 1301–3. doi: 10.1126/science.1127704.

Yoneyama, S., Y. Guo, M. B. Lanktree, M. R. Barnes, C. C. Elbers, K. J. Karczewski, S. Padmanabhan, et al. 2014. "Gene-centric meta-analyses for central adiposity traits in up to 57 412 individuals of European descent confirm

known loci and reveal several novel associations." *Hum Mol Genet* 23 (9): 2498–510. doi: 10.1093/hmg/ddt626.

Yu, J., X. Lan, X. Chen, C. Yu, Y. Xu, Y. Liu, L. Xu, H. Y. Fan, and C. Tong. 2016. "Protein synthesis and degradation are essential to regulate germline stem cell homeostasis in *Drosophila* testes." *Development* 143 (16): 2930–45. doi: 10.1242/dev.134247.

Yu, J., M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, et al. 2007. "Induced pluripotent stem cell lines derived from human somatic cells." *Science* 318 (5858): 1917–20. doi: 10.1126/science.1151526.

Yue, Z., J. Xie, A. S. Yu, J. Stock, J. Du, and L. Yue. 2015. "Role of TRP channels in the cardiovascular system." *Am J Physiol Heart Circ Physiol* 308 (3): H157–82. doi: 10.1152/ajpheart.00457.2014.

Yurkovic, A., O. Wang, A. C. Basu, and E. A. Kravitz. 2006. "Learning and memory associated with aggression in *Drosophila melanogaster*." *Proc Natl Acad Sci U S A* 103 (46): 17519–24. doi: 10.1073/pnas.0608211103.

Zalucki, O., and B. van Swinderen. 2016. "What is unconsciousness in a fly or a worm? A review of general anesthesia in different animal models." *Conscious Cogn* 44:72–88. doi: 10.1016/j.concog.2016.06.017.

Zehring, W. A., D. A. Wheeler, P. Reddy, R. J. Konopka, C. P. Kyriacou, M. Rosbash, J. C. Hall. 1984. "P-element transformation with *period* locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster*." Cell. 39 (2, pt. 1): 369–76. doi: 10.1016/0092-8674(84)90015-1.

Zemelman, B. V., G. A. Lee, M. Ng, and G. Miesenböck. 2002. "Selective photostimulation of genetically chARGed neurons." *Neuron* 33 (1): 15–22. doi: 10.1016/S0896-6273(01)00574-8.

Zhang, S. D., and W. F. Odenwald. 1995. "Misexpression of the *white* (*w*) gene triggers male-male courtship in *Drosophila*." *Proc Natl Acad Sci U S A* 92 (12): 5525–29. http://www.pnas.org/content/92/12/5525.

Zhang, S. L., A. V. Yeromin, X. H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K. A. Stauderman, and M. D. Cahalan. 2006. "Genome-wide RNAi screen of Ca(2) influx identifies genes that regulate Ca(2) release-activated Ca(2) channel activity." *Proc Natl Acad Sci U S A* 103 (24): 9357–62. doi: 10.1073/pnas.0603161103.

Zhang, Y. V., R. P. Raghuwanshi, W. L. Shen, and C. Montell. 2013. "Food experience-induced taste desensitization modulated by the *Drosophila* TRPL channel." *Nat Neurosci* 16 (10): 1468–76. doi: 10.1038/nn.3513.

Zhou, C., R. Franconville, A. G. Vaughan, C. C. Robinett, V. Jayaraman, and B. S. Baker. 2015. "Central neural circuitry mediating courtship song perception in male *Drosophila*." *Elife* 4:e08477. doi: 10.7554/eLife.08477.

Zhou, X. L., A. F. Batiza, S. H. Loukin, C. P. Palmer, C. Kung, and Y. Saimi. 2003. "The transient receptor potential channel on the yeast vacuole is mechanosensitive." *Proc Natl Acad Sci U S A* 100 (12): 7105–10. doi: 10.1073/pnas.1230540100.

Zikova, M., J. P. Da Ponte, B. Dastugue, and K. Jagla. 2003. "Patterning of the cardiac outflow region in *Drosophila*." *Proc Natl Acad Sci U S A* 100 (21): 12189–94. doi: 10.1073/pnas.2133156100.

Zimmer, C. T., W. T. Garrood, A. M. Puinean, M. Eckel-Zimmer, M. S. Williamson, T. G. Davies, and C. Bass. 2016. "A CRISPR/Cas9 mediated point mutation in the alpha 6 subunit of the nicotinic acetylcholine receptor confers resistance to spinosad in *Drosophila melanogaster*." *Insect Biochem Mol Biol* 73:62–69. doi: 10.1016/j.ibmb.2016.04.007.

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