The Origin and Organization of the Bee Colony *Apis mellifera L.*

Eugeney Eskov

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INTRODUCTION

In the animal kingdom and in the insect class, the honey bee (*Apis mellifera L.*) is distinguished by the specificity of its lifestyle and the variety of its adaptations to a wide range of habitat conditions. Differentiation into subspecies and the evolutionary plasticity of the species is associated with the diversity of the climatic conditions of the habitation area. G.K. Gotze (1954) and Yu. A. Pesenko et al. (1989) distinguished eight subspecies of the honey bee: the Western Mediterranean (*A. m. intermissia* But.–Reep., 1906), the Euro-Siberian (*A. m. mellifera* L., 1761), the Middle Eastern (*A. m. meda* Skov., 1929), the Afro-Tropic *(A. m. adansonii* Latr., 1804), the Cape (*A. m. capensis* Esch, 1822), the East North African (*A. m. lamarckii* Cock, 1906), the Madagascan (*A. m. unicolor* Latr., 1804) and the Caucasian (*A. m. remipes* Gerstacker, 1862).

Their eurybionticity allowed the honey bee to occupy a wide area that extends to all inhabited continents. The progressive evolution of the species is associated with the development of sociality and the transformation of the colony into an evolving biological unit. On this basis, the reproduction and resettlement of bee colonies have developed through sociotomy, which has some distant resemblance to asexual reproduction in protozoa.

Human interest in bees goes back to antiquity. Settlements of bees attracted people with an abundance of carbohydrate (honey) and protein (pollen and brood) food reserves contained in them. However, in order to use them, it was required to obtain such necessary skills as detecting bee colonies and extracting bee products from their dwellings, which is life-threatening because bees have an effective means of protection – a poisonous sting.

Initially, the extraction of bee products by man was one of the forms of hunting for bees, as well as for many wild animals. As the methods of hunting improved, the bee trade began to appear. Its improvement was stimulated to a large extent by the difficulties associated with the detection of bee colonies, and intense competition between hunters. Also, animals were competitors for hunters, since they were attracted by honey or the bees themselves.

Bee trading in various forms existed and progressed for several thousand years. Honey hunting (honey harvesting) is one of its forms. At the initial stages of its development, it already included elements of bees' care, which was expressed in the preparation of places for the settlement of swarms. For these purposes, trees were hollowed out with special cavities.

The use of smoke is one of the earliest methods mastered by man for controlling the behavior of bees. According to the cave paintings of the Mesolithic era, preserved in the caves of Eastern Spain, people started to use smoke at least 7–9 thousand years ago to suppress the aggressiveness of bees. Even nowadays this instrument is used by beekeepers. But bees associate the smoke not with a beekeeper but with fires that induce bees to fill their honey crops. This prevents the bees from bending their abdomen, which is necessary to sting the beekeeper.

A significant contribution to the improvement of beekeeping technology was made by the invention of the artificial wax foundation for the construction of honeycombs (honeycomb base) by G. Kretchmer in the first half of the nineteenth century. The use of a perforated artificial wax foundation made it possible to control the construction of honeycombs with given cell sizes. As a result, the bees were able to reduce energy costs for the construction of cells.

Despite the use of bees in the household, the honey bee did not acquire the typical traits of a pet. Their domestication effected a change in their morphophysiological features and the development of specific forms of behavior that are controlled by a person when their normal existence is strongly dependent on the human. In contrast, different technologies for keeping and breeding bees did not affect the modification of the genetically programmed behavior of bees.

The preservation of the typical honey bee's species characteristics that have not changed in the process of long-term domestication does not allow one to consider it as a typical pet. The honey bee kept in the household remains a wild animal, capable of independent existence regardless of a person's influence. In the process of sociotomy, bee colonies leaving their hives successfully master new dwellings and continue their independent existence regardless of humans. An increase in the number of bee colonies and their expansion through the development of new zones with temperate and cold climates occurred in the evolution of a species in this way. Natural selection favored the honey bee's development of instincts for reproduction and settlement.

CHAPTER ONE

THE ORIGIN OF COLONIES OF APIDAE

In the group of aculeate Hymenoptera (Aculeata), bees represent one of the largest superfamilies of Hymenoptera formed in the process of coupled evolution with flowering plants. Since the flourishing of angiosperms occurred in the Late Cretaceous, the appearance of bees probably dates to this epoch (Michener, 1965, 1975; Rasnicin, 1980a, 1980b; Brothers, 1975; Dietz, 1986).

The number of bee species increased progressively at the end of the Oligocene and the beginning of the Miocene (Rasnicin, 1980a; Michener, 1975, 1979). The superfamily includes 11 families, uniting 520 genera. Nowadays, the greatest taxonomic diversity is in the Neotropical, Neo- and Palearctic realms, where 315, 260 and 243 genera of bees are known respectively. Their lowest representation is in the Australian region, where only 18 genera are found (Radchenko & Pesenko, 1994).

Bees have developed care for their offspring. The expression of this instinct depends on their way of life. Bees are divided into solitary, social and parasitic living by this feature. Most species of the modern superfamily of bees are represented by solitary living insects. Various forms of social organization are found in representatives of the Apidae, Bombidae, Halictidae, Anthophoraidae, and Euglossiedae families.

In the order of the Hymenoptera, social insects are found not only among bees. Fully social species are represented by the superfamily of Formicoidea (ants). Among the other 35 orders of insects, only the representatives of Isoptera (termites) lead a social way of life. Unlike the Hymenoptera, this order is completely represented by socially living species.

The presence among bees of a variety of transitional forms from typical solitary-living to highly organized socials allows us to trace their general patterns of origin and sociality development. The main developing directions can be traced by comparing the advantages in the struggle for existence and the social relations of modern species of bees that differ in their way of life.

It is possible to distinguish several levels of social organization. Eusociality is considered to be the rudimentary (initial) form of social organization. This is expressed by the fact that the female offspring of the female-foundress can provide her with some assistance in life support in the temporary colony. When it breaks up the generation of females flies away and establishes its own nests. Temporary colonies also form subsocial species. A similar organization is characteristic of eusocial species. Highly organized eusocial species are those that constantly live in colonies or where at least two generations of the female-foundress live with her in one nest. Adult individuals participate in the construction and protection of the nest, the delivery of food and the feeding of the female-foundress' offspring. Along with the female-foundress, her daughters can also participate in the reproduction of offspring.

Nest Constructions and Trophic Provision of Offspring

Solitary bees. The nest construction of solitary bees depends on the place of settlement. Most often they use different cavities in plants, rocks or the ground. Bees subject these cavities, used for sheltering offspring, to some processing (deepening, expansion, cleaning). Many species settling in the ground dig out passages and cavities by themselves, while some build nests in open spaces (stones, tree trunks, soil surfaces, etc.). The construction of nests is carried out by females (female-foundresses) that are ready to perform reproductive functions. The choice of the place of settlement, materials for the construction of shelters and ways to process them belong to species-specific features.

Among a wide range of nest structures, the most primitive ones are those where food stocks and developing individuals are in one common cavity. That is typical of the bee *Metallinella atrocaerulea* (Fig. 1A). It settles in a ready shallow cavity in wood that has one inlet. The female fills the nest cavity with food (pollen mixed with nectar), ovipositions 4–12 eggs in it and then seals the entrance with a plug of crushed leaves. The emergence of larvae and their development does not occur simultaneously. The first to complete the larval stages and turn into pupae are individuals emerging from the eggs ovipositioned at the base of the nest; at the entrance of the nest settle the latter – at the upper part from the facing side of the plug (Radchenko, 1979). There is competition between the larvae due to the localization of all of them in the same trophic substrate, whose intensity of consumption depends on their age. This prevents the appearance of consolidation among developing individuals.

However, in the superfamily of bees, the use of a common nesting chamber by developing individuals has a limited occurrence. Along with *M. atrocaerulea*, this form of offspring reproduction was found in the family of Anthophoridae in representatives of the genus *Allodapini* (Radchenko, Pesenko, 1994). The insignificant representation of species with this form of nesting in the rapidly progressing superfamily of bees is evidently associated with the incipience of rather perfect instincts of caring for offspring, based on the use of the individual isolation of developing offspring.

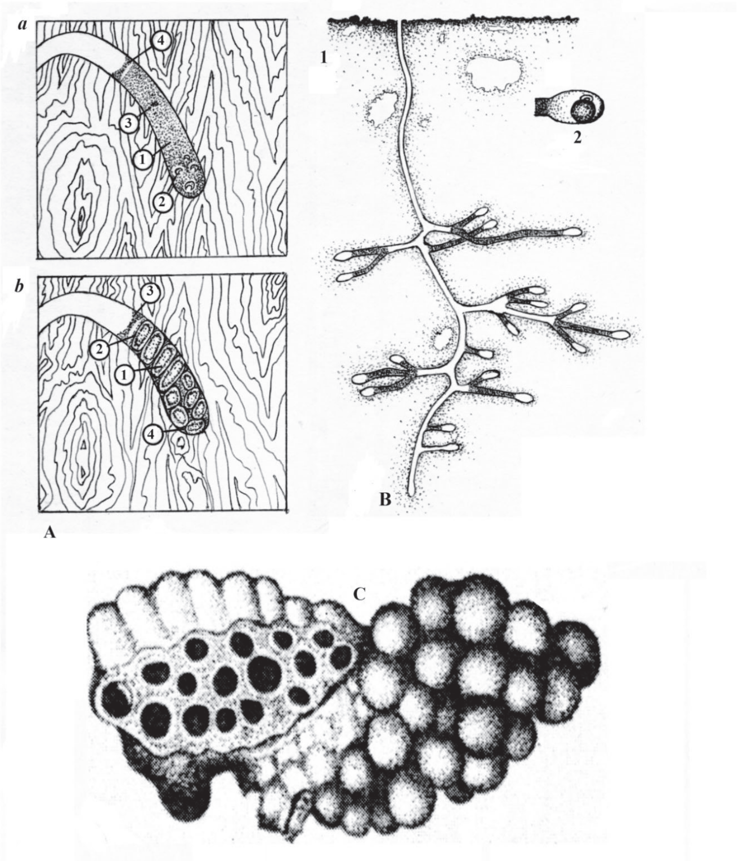

Fig. 1. Nest structures of solitary living bees: A – a nest of *Metallinella atrocaerulea* with developing bees: a-bees at the egg stage and larvae (1-pollen; 2-larva; 3-egg; 4-plug); b-stage of pupae (1-cocoon, 2-excrement, 3-plug, 4-formed cocoon) (by: Radchenko, 1978); B – *Nomioidea minutissimus* (1) and one of the sealed cells with food and eggs (2) (by: Radchenko, 1979); C – ground honeycomb of the bee *Halictus quadricinctus* (by: Blagoveshchenskaya, 1983)

Natural selection in bees favored the acquisition of instincts responsible for behavior providing individual isolation to the developing offspring. This was fully satisfied with the construction of cells where only one individual could develop. It was also specially supplied with food once or periodically during its development. In such situations, the cell turned into an elementary unit of nesting construction (Fig. 1B, C).

The choice of the settlement's location and the materials used for the construction of the cells differ in diverse species of bees with a wide variety. Many types of Megachilidae construct cells of a cylindrical shape (or close to such a form) from pieces of leaves or petals of flowers. Species' differences determine the size of the body of insects, which affects the size of the cells. The discrepancy between the large diameter of the nest cavity and the biological needs of the individuals developing in them is corrected by an increase of the walls' thickness. However, the reduction in the diameter of the cells constructed in narrow nest cavities is reflected in the decrease in size of the developing bees. Therefore, improving the nest behavior of Megachilidae was accompanied by the acquisition of the ability to control the size of the cavity chosen for settlement. Its successful choice is achieved by reducing the energy costs for the construction of cells by reducing the walls' thickness under optimal development conditions. Thus, with the possibility of choice, leafcutter bees (*Megachile rotundata*) prefer a diameter of tunnels limited to 6–6.5 mm for settling (Pesenko, 1982).

The number of cells constructed in the nest cavity depends on its depth and is limited by the reproductive potential of the female-foundresses. The reproduction of the leafcutter bee is limited to about 40 offspring. Within a day the female can oviposition no more than two eggs. This limits the breeding rate of offspring (Stephen et al., 1969).

The leafcutter bee fills each cell with the pollen mass, lays an egg on it, and then closes (seals) it with the cover. Especially for this, the bee spends up to 6 pieces of leaves of the same plants from which the cell is built. The constructed nest can contain 1–20 cells and is completed with the construction of the plug. The bee spends 8–50 pieces of leaves on it. A small cavity usually remains unoccupied between the plug and the closest cell cover (Stephen, Torchio, 1961). The plug performs a protective role, reducing the negative impact of external environmental factors and prevents penetration into the nest by other insects (Stephen et al., 1969).

The ratio between females and males that develop in the same nest depends on the size of the cells, their localization in the nest cavity and the food supply. In addition, the female-foundress ovipositions the fertilized eggs from which the females develop into the cells that appeared at the beginning of the nest’s construction, and the unfertilized ones closer to the exit (which were constructed later). Developing females are supplied more abundantly with food than males, who leave the nest before females (Stephen et al., 1969; Pesenko, 1982). For females, it takes a longer time to develop from egg to adult stage than for males.

The use of plant leaves by Megachilidae and other species of solitary bees for nesting is conditioned by their easy accessibility, but is limited by their low strength and high moisture permeability. This is due to the acquisition by the bees of the tools that contributed to increasing the protection of developing offspring from the adverse effects of the physical and biotic factors of the environment. On this path, instincts and morphophysiological adaptations have been developed that ensure the expansion of the diversity of settlement places. Thus bees of the genera *Heriades (Osmiini)* and *Megachile* cover the inner surfaces of the cells with resinous substances. This results in an increase in the waterproofing of the cells and protection from the penetration of parasites in it (Tsuneki, 1970; Maciel, 1976).

There are objects used by some bees that, without additional processing, meet the needs of developing individuals in the natural environment. For example, *Osmia rufonirta* and *O. bicolor* (Megachilidae) settle in the empty shells of snails in order to use each of them for one developing individual. To do this, the bee firstly cleans the shell, then brings the food into it, and after ovipositioning the bee seals the egg with the paste made from chewed leaves. Further care for the offspring is expressed in the sheltering of shells from competitors and enemies as well as from overheating by the sun's rays. *O. rufonirta* usually roll the shell from an open surface into a thicket of grassy vegetation and *O. bicolor* dig it into the soil (Grozdanic, 1971).

The strengthening of competition for suitable ground nesting places could affect the acquisition of instincts and morphophysiological adaptations for settlement in the ground. First of all, bees could be attracted by cavities in the dead parts of the root system that emerged on the surface as well as tunnels and depressions left by soil invertebrates in the ground. So, *Megachile albocincta* adapted to use earthworms’ tunnels on the surface for nesting. As material for cell building, the bee uses pieces of leaves. The construction of the first cell begins at the depth of the tunnel and the last one at its exit. This behavior is similar to the leafcutter bee that settles in terrestrial cavities (Fabre, 1963).

The increase in the adaptability of bees to settlement in the ground developed in the direction of growing independence from the availability of suitable cavities and environmental factors that threaten the developing offspring. In this way, bees have acquired devices for building passages and cavities in the ground. The form of settlement when the female-foundress digs out a small hole in the ground where it is possible to build at least one cell belongs to the simplest one. Such nest behavior is characteristic of one species of Colletidae – *Colletes daviesanus* – settling on sandy, forest or limestone unshaded hillsides with southern exposures. In the excavated holes, the bee constructs up to four cells singly or in small rows (Scheloske, 1974). The representative of another Megachilidae family – *Megachile japonica* – constructs up to eight nests containing only 1–2 cells (Maeta, 1979). Each of them is dug out with a cavity about 2 cm in depth.

The complication of the nest behavior of bees settling in the ground was expressed in the acquisition of instincts for the construction of tunnels, labyrinths and brood chambers. Relatively simple nests of this type are built by the bee *Cantridini aethyctera* (Anthophoridae). It settles in dry forest soil and digs an almost vertical tunnel with a depth of 8–14 cm, to which 3–6 cells are adjoined (Vilson, Frankie, 1977). Side branches from the entrance tunnel to horizontally located brood cells are constructed by the bees *Panurginus atriceps*, *P. occidentalis* (Andrenidae) (Rust, 1976) and *Nomioides minutissimus* (Halictidae) (Radchenko, 1979). In *Augochlorella dentata* (Halictidae), a bunch-like cluster of cells strengthened on ground supports have a remote resemblance to honeycomb (Eickwort G., Eickwort K., 1973). The ground nest of the Halictus bee (*Halictus guadricinctus*) is closer to them. This bee has a nest with a convex or spiral curved structure measuring approximately 6x12 cm that is formed by several tens of closely spaced cells (Blagoveshchenskava, 1983).

A threat to the developing offspring of bees is represented by a hydro factor in many environmental situations. The development of protection means against it involves the acquisition of waterproofing instincts of the cells' contents. Bees use plant extracts or their own secretions as moisture-proof substances. Using the pitch of woody plants, the bee *Melipona tourea* (Anthophoridae) veneer the walls of brood cells. The surface of the cells is covered with an oral secretion of the *Nomia melanderi* (Halictidae) settling in alkaline soil (Batra, 1970). The secretion of the Dufurov gland, which is a complex mixture of liquid triglycerides, is used to cover the cells of the Anthophora bee *Anthophora antiope* (Batra, 1980) and *A. abrupta* (Norden et al., 1980).

The high reliability of a cell's contents in isolation is provided by wax. It is produced in bees by the glands of the body's integument. The use of wax or wax-like substances has been found in solitary living Andrenes *Andrena ovatula* (Wafa et al., 1972), Anthophoras *Anthophora urbana*

(Mayer, Johansen, 1976), Ptilothrix *Ptilothrix bombiformis* (Rust, 1980), etc. These substances usually cover only cell walls, and the plug is usually constructed from the nest substrate. For example, *Andrena urbana* seals the cell with a soil plug (Mayer, Johansen, 1976).

Nesting in open surfaces is the result of the absence of suitable cavities, morphological devices for processing natural substrates or their physical properties (high strength, humidity, etc.). A widespread use of such building materials as small stones fastened with dust and moistened by oral secretions was seen in the bees *Chalicodoma pyrenaica* (Fabre, 1963), *Hoplitis anthocopoides* (Eickwort G., Eickwort K., 1973), *Osmia anthocopoides* (Radchenko, Pesenko, 1994), etc. The female firstly supplies each of the alternately constructed cells with food, then ovipositions an egg in and seals it. The material used to seal the cell does not contain small stones, as they could prevent the release of individuals reaching the imago stage. The construction of such nests containing a little more than ten cells is completed by the building of an additional shelter above them.

Eu- and subsocial species. Primitive social insects include bumblebees (*Bombus*), represented in the insects' fauna by about 250 species. In bumblebees living in the Palearctic, the overwintered female-foundress during the spring-summer season creates a colony that breaks down in late summer or early fall. Burrows of rodents, tree hollows, and crevices of rocks are used as places for bumblebees to settle. The bumblebee nest is covered with various heat-insulating materials found nearby such as dry moss, straw, leaves, etc. A wax-like shell can be used for the waterproofing of the nest (Eskov, 1992; Eskov, Dolgov, 1986). Species living in the tropics use the live or dead leaves of plants to shelter the nests (Janzen, 1971) when they settle on trees.

The seasonal cycle of the development of the bumblebee colony begins with the search for a suitable nesting place for the fertilized female, where she constructs a cell for feed and a brood chamber for her offspring (Fig. 2). Wax is used as a building material by bumblebees and is usually mixed with pollen. This building material is used many times in their nests and when they settle in the abandoned nests of other colonies (Alford, 1971). It is also possible to use other wax-like substances as a building material, for example, plasticine has been found in nest cavities. If there are small containers resembling feed cells, bumblebees can finish building them and store their food reserves there (Dolgov, 1982).

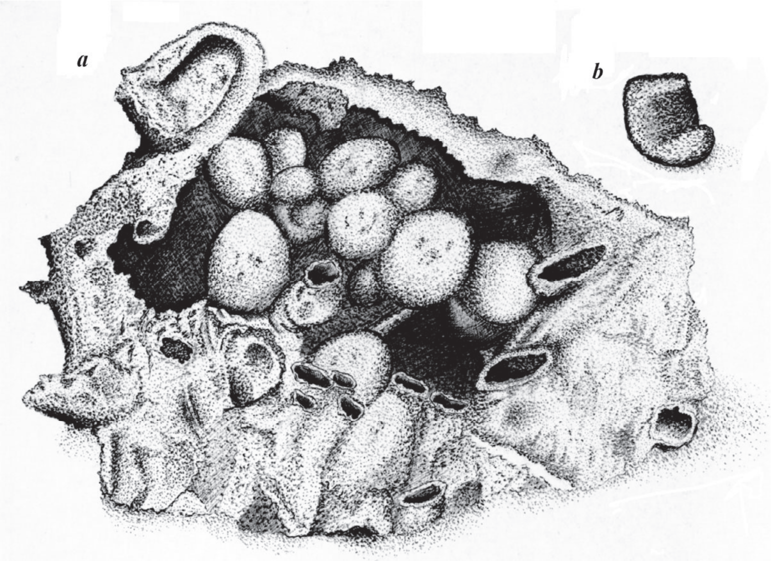

Fig. 2. Dissected nest of the bumblebee *Bombus terrestris* (a) and an enlarged cell with sealed eggs (b).

The construction of the brood cell by the female-foundress is preceded by the detachment of the wax base. The female forms a pollen ball moistened with nectar and rings it with a small wax roller on it (Dolgov, 1982). The females of *B. pratorum*, *B. humilis*, *B. pascuorum* and others make holes in the pollen ball where one egg is ovipositioned (Alford, 1975). The female *B. hortorum* ovipositions eggs at different levels of the pollen ball (Alford, 1971). Females of *B. balteatus* and *B. polaris* oviposition eggs in pollen balls, fill them with a pollen mixture and then cover them with a waxy membrane (Alford, 1971, 1975; Richards, 1973). The construction of separate brood cells where the female ovipositions one single egg is characteristic of *B. rufocinctus* (Hoobs, 1965).

Different types of bumblebees oviposition 8–16 eggs in the first brood cell. Their number also varies among females of the same species. A different time is required for the female-foundress to complete the first ovipositioning cycle. Under favorable weather conditions, *B. agrorum* spends about three days ovipositioning eggs (Free, Butler, 1959; Alford, 1971, 1975; Dolgov, 1982).

The emerging larva systematically receives food (nectar and pollen). It is brought into the cell through the pollen pocket or temporary holes made in the cell (Sakagami, Zucchi, 1965; Alford, 1971; Sakagami, 1976). As the larva grows, the female repeatedly rebuilds the brood cell, completing the waxy membrane. Subsequent brood cells are usually placed randomly on a common base constructed at the initial stages of the nest's construction. With the emergence of working individuals, the colony's protection from the adverse effects of biotic and abiotic environmental factors and the reproduction of offspring increases.

Eusocial species. In highly organized social types of bees, nest structures mainly differ in the use of building materials, the orientation of honeycombs and the location of fodder stocks. According to these species' features, the greatest differences are found between stingless bees and representatives of the genus *Apis*.

Stingless bees. A large group of stingless bees (Meliponinae) permanently live in colonies where structural organization and size can vary significantly. For the place of settlement, stingless bees usually use various natural shelters such as trees or ground cavities and also inhabited or abandoned termite mounds (Wille, Michener, 1973). Some species settle in trees (Darchen, 1969).

Regardless of the settlement place, bees isolate the nest from the outside environment with a multi-layered shell using wax, pitch, clay, wood and other materials (Koeniger, 1976). The entrance to the nest is in the form of a slit or tunnel that in *Meliponen* is made of clay, and in *Trigona* – of resin (Brian, 1986). In order to protect against the penetration of robbers and parasites into the nest, the bees guard the entrance during the day, and at night close it with a cork constructed from the material used for the building of the tunnel entrance. Some species protect the entrance from ants with sticky substances (Ihering, 1903; Rooley, Michener, 1969). The drainage pipe can be constructed to remove water in the lower part of the nest (Brian, 1986; Michener, 1974). Some wax balls may be used in the nest as a reserve building material (Brian, 1986).

The cells for brood and food, as a rule, differ in shape and location in the nest. The largest cells are constructed to store the reserves of honey and pollen. They are placed on the periphery of the nest. In *Melipona beecheii*, their diameter reaches 2–3 cm (Darchen, Delage-Darchen, 1975). The main part of the nest cavity is occupied by relatively small cells for the brood. They are strengthened by supports. The nest material is used for these constructions. In some species the brood cells are arranged in groups forming bunches. For most species it is characteristic to have a horizontal distribution of brood cells, forming one-sided honeycombs (Fig. 3). The construction of vertical two-sided honeycombs was found in *Dactylurina staudingeri*. Working individuals and drones are developed in the cells of honeycombs. Cells for the reproduction of large females (queens) are located at the bottom of the honeycomb or in the inner surface of the shell of the nest (Michener, 1974).

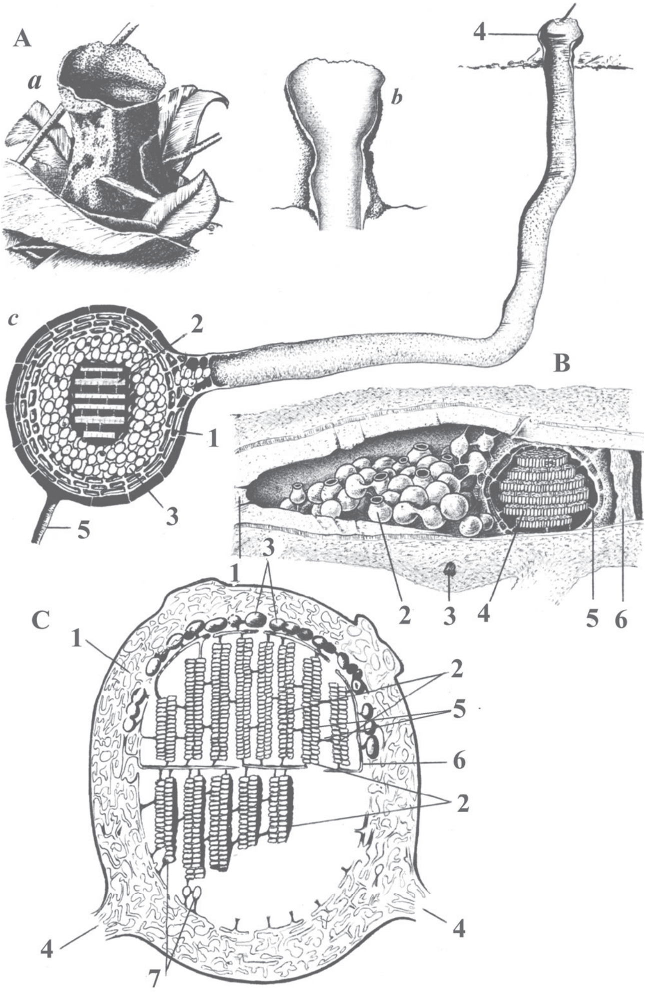

Fig. 3. Nesting constructions of stingless bees (by: Michener, 1974): A – *Trigona recursa* nest in the ground: a – the appearance of the entrance; b – entry section; c – longitudinal section of the nest located in the ground (1 – layered shell, 2 – honeycombs, 3 – fodder reserves, 4 – entrance, 5 – drainage tube); B – nest in the hollow of a tree (1 – wall that limits nesting space, 2 – fodder reserves, 3 – entrance, 4 – brood honeycombs, 5 – protective shell, 6 – wood); C – section of the nest of *Dactylurina staudingeri* (1 – nest shelter, 2 – brood cells, 3 – fodder reserves, 4 – entrance to the nest, 5 – supporting partitions, 6 – drainage tubes).

Working individuals are engaged in the construction of the nest cavity and cells. They also fill the cells with food (a mixture of pollen and nectar), adding to it the secretion of hypo- or propharyngeal glands. Firstly the queen consumes part of this food and then ovipositions an egg. After that, bees seal the cell, not worrying about the trophic supply of the developing individual (Darchen, Delage-Darchen, 1975; Sommeijer et al., 1982).

Bees of the genus Apis. The genus Apis includes three subgenera, each of which unites two species that have similarities in their lifestyle in the specificity of settlement places and the design of nesting structures. The most evolutionarily advanced subgenus combines *A. mellifera* and *A. cerana*. The intermediate position is taken by the species *A. dorsata* and *A. laboriosa* (subgenus *Megapis*). The most primitive species are *A. florea* and *A. andreniformis*, which form a subgenus (*Micrapis*). The most widespread is *A. mellifera*. Other species mainly live in the tropical forests of Southeast Asia. Only *A. cerana* inhabit the Palearctic (in Northeast China, Japan, and Southern Primorye) and *A. laboriosa* live in the Himalayas, settling at an altitude of 3–4 km above sea level.

Representatives of *Megapis* and *Micrapis* construct only one honeycomb attached to a tree branch, the bottom surface of a rock or other aboveground objects. When choosing a settlement place, *Microapis* bees must see at least a small part of the sky from a honeycomb. By its polarization, bees determine the position of the sun; this is used in the system of spatial orientation. This is not required for the other four species of bees of the genus *Apis* (Lindauer, 1971; Eskov, 1979, 1992).

Bees start the construction of honeycombs at the place where it is attached and complete it later in the process of mastering the new settlement place. *A. florea* usually settles on the side of rocks facing to the south in the summer and to the east in winter (Dutton, Simpson, 1977). Its honeycombs usually have a shape close to a semicircle or an ellipse with one side having an area of about 500 cm². The dimensions of the cells differ depending on their purpose. Relatively small cells with a diameter of about 2.5 mm occupying approximately 80% of the honeycomb's surface are used for the development of working individuals. Larger cells are used for the development of drones. In the upper part of the honeycomb, there are large cells that are used for storing honey and flower pollen. In the lower part of the honeycomb, bees build several cells of an acorn-form shape where queens develop. When settling on tree branches, the nest's protection from ants is achieved by constructing rings made of sticky resinous plant extracts (Butler, 1969).

When settling on trees, bees of the subgenus *Megapis* build honeycombs at a height of up to 20 m, and less often of up to 40–80 m above the surface of the soil. The honeycomb can hang under the weight of honey close to the ground but usually does not come into contact with it. The clustering of nests in one place is characteristic of these bees. More than 150 colonies can settle on one large tree (Butani, 1950), although there are also single nests. The minimum diameter of the branches used to build the honeycombs is 12 cm. However, most often, bees prefer branches with a diameter of about 30 cm (Morse, Laigo, 1969). When the construction is completed, the honeycomb becomes a truncated ellipse where one side's area can reach 0.5–1.5 cm^2 (Kaiser, 1976).

The largest cells (their depth can exceed 8 cm) localized at the top of the honeycomb are used as food stocks (Singh, 1962; Morse, Laigo, 1969). Cells used for the development of working individuals and drones come under the food tier. In this part, the thickness of the honeycombs decreases to 35-40 mm. Ellipsoidal queen cells are located in the lowest part of the honeycombs (Morse, Laigo, 1969).

Honeycombs' protection from the direct influence of biotic and abiotic factors is provided by bees forming a multilayer covering with their bodies. This is mainly formed by young bees hanging upside down, clinging to each other with their legs (Roepke, 1930). The size of such a nest shelter, which usually hangs over the lower border of the honeycomb, depends on the number of bees in the colony that reaches its maximum before the sociotomy (Morse, Laigo, 1969).

The bees *A. mellifera* and *A. cerana* differ from the bees *Micrapis* and *Megapis* in their adaptation to sheltered settlements (hollows of trees, clefts of rocks and other cavities). Such shelters protect them from the influence of unfavorable physical and biotic factors. When it is possible to choose a place of settlement, the honey bee prefers cavities in wood with a volume of 70 ± 103 cm^3 located at an altitude of 8–19 m (Petrov, 1983). This indicates that they have instincts of evaluating the suitability of a potential dwelling with the biological needs of the colony.

The nests of Indian honey bees form vertical two-sided honeycombs, the number and shape of which depend on the configuration of the nest cavity. One large honeycomb can be enough for a nest of honey bees that has settled in a narrow cavity. In representatives of this species, as well as other bees of the genus *Apis*, the upper part of the honeycomb is usually used for food stocks. The brood cells are placed below, and the acorn-form queen cells – on the periphery. At different times, all honeycomb cells can be used to store food except for the queen cells.

The diameter of the cells (the distance between opposite angles) in a honey bee nest varies from 4.1 to 7.7 mm (Fig. 4). The cells with a diameter of 5.1-5.4 and 6.5-7.0 mm have the largest frequency of occurrence (about 60%). The first of them are used for the reproduction of working individuals, the others – for drones. The tendency to cell enlargement can be traced at the initial stages of honeycomb construction during the period of mastering a new dwelling (Eskov, Eskova, 2001, 2012).

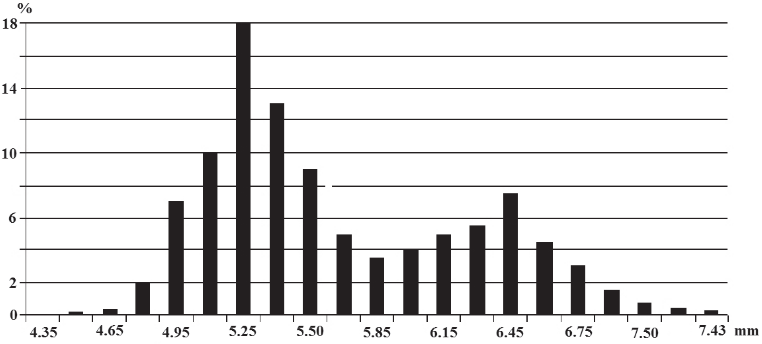

Fig. 4. Percentage distribution of 43,279 cells (y-axis), differing in the distance between their opposite angles (x-axis); honeycombs are built up by bees in free nesting spaces.

Preserving the building material and maximizing the use of the nest structure in bees of the genus *Apis* favored the closeness of cells and the honeycomb formation. The bees built bunch-shaped clusters of cells in the initial phases of the development of this building instinct that is typical for some Halictus and relatively low-ordered stingless bees. One-sided honeycombs are distinguished by higher ordering. They are widespread in stingless bees and are also found in some social types of Halictus. At the same time, the needs of relatively small Halictus colonies for the reproduction of offspring are satisfied by the presence of only one honeycomb. However, this is not enough for large colonies of stingless bees. Therefore, they had a need for constructing a multi-tiered nesting structure containing several honeycombs connected by a plurality of supports and bridges. They provide an increase in the strength of the brood nest zone, but this is associated with additional costs to building materials and the restriction of the useful volume of the nest cavity. Cells of one-sided honeycombs of stingless bees are unsuitable for food stocks. For these purposes, large cells that are tuned outside the brood zone are used.

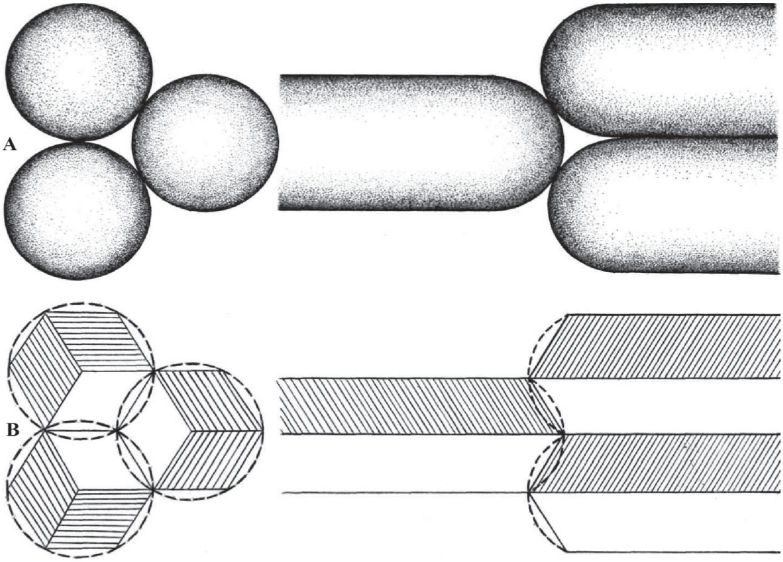

Fig. 5. Schematic representation of the transformation of rounded cells into hexahedral cells due to their compaction (A), and the resulting transformation of oval bottoms into trapezoidal ones (B).

The transition from a horizontal to a vertical placement of cells is associated with a modification of building instincts, which could be motivated by the adaptation to shelter life. With the development of the instinct for two-sided honeycomb cells' construction, the selection favored the convergence of the separated common adjacent walls. The partial overlapping of their bases on opposite sides of the cells is associated with the convergence of cells. Each of the three converging cells on one side of the honeycomb forms a concave base for the cell on its opposite side. The maximum convergence of cells led to the transformation of their originally rounded shape into a hexagonal one, and the combined parts of the bases transformed into trapezoids. Each of the three rhombs forming the base of the cell began to be included in three adjacent cells on the opposite side, thus maximizing the convergence of the cells of bilateral honeycombs, saving building material (wax) and providing a relatively high amount of strength (Fig. 5).

Morphofunctional Differentiation of the Bee Colony

The size and the members of the colony. Colonies of eu- and subsocial species include a small number of adults and developing individuals. In the nests of *Losioglossum sp*. (Halictidae), during the intensive development of the colony, there may be 20–25 adult females, approximately the same number of developing individuals at the larval and pupal stages, and about 10 males (Houston, 1970). *Exoneura eremophila* and *E. setosa* (Anthophoridae) constantly reproduce males during the period of the colony's existence, but they do not stay in the nest for a long time. The maximum number of adult females in the nests of these species can reach 20 individuals, and the brood – a little more than 40 cells (Houston, 1977).

The number of bumblebee colonies has a pronounced tendency to increase with the migration of various species from zones of cold and temperate climates to subtropical and tropical ones. Short summers in the upper latitudes limit or completely prevent the raising of workers. Therefore, with a move to cold climate zones, bumblebees come closer to the way of life of typical solitary living insects (Malyshev, 1928; Richards, 1955a). In contrast, in the tropics, the raising of working individuals, mature females, and males is possible throughout the year (Michener, Amir, 1977).

In seasonal colonies of species inhabiting temperate climates, by the time of maximum development, the number of adults is usually limited to a few tens, and in the tropics – by hundreds. Among adults in tropical species, the ratio between mature females and working individuals is maintained at about 1:10 (Ihering, 1903). In seasonal colonies, females are generally reproduced more than males. For example, during years with warm weather, by the end of summer in *B. agrorum* colonies the ratio of the reproduction of females and males corresponds to about 0.6:1, and at the early onset of cold weather this changes to 1:1.6 (Brian, 1951, 1952). The number of males also increases in cases of the loss of the female-foundress. In *B. terricola* and *B. melanopygus*, the ratio between females and males can be as high as 1:6 in size, and 1:2.9 in biomass (Owen et al., 1980). The quantity of males in the colony increases correspondingly to the increase of the period between the onset of ovipositioning by the female-foundress and the working individuals of haploid eggs (Duchateau et al., 2004).

Interspecific (interspecies) differences in stingless bees are characterized by high variability. The smallest number of them is several hundred individuals. This is characteristic for colonies of *Melipona quadrifasciata*, *M. scutellaris*, and *Trigona silvestris*. One to several thousand is characteristic for colonies of *T. capitata*, *T. mombuca*, *T. testaceicornis*, *T. droryana*, and *T. Postica*. 50–180 thousand adults can be contained in colonies of *T. rufrus* (Lindauer, Kerr, 1960). The ratio between the number of queens and working individuals in them is usually in the range of 1:3–1:4 and can exceed these limits depending on the availability of food for colonies (Darchen, Delage-Darchen, 1975). When there is a lack of food in *M. beichei* colonies, this ratio increases up to 1:9, and with an abundant food supply it can reach 1.3:1 (Darchen, 1973).

In the colonies of the honey bee, the workers usually have the largest representation. Their quantity is subject to pronounced seasonal variability depending on the external temperature and productivity of the forage place. In zones with a temperate and cold climate, the maximum numbers of worker bees reach 30-70 thousand individuals in the summertime, and the minimum – at the end of wintering and the resumption of flying activity. Drones are temporary members of the bee colony. They emerge in April-June and are expelled from colonies in late summer or early fall. Drones remain in the nests for the winter only in queenless colonies.

The honey bee is characterized by a monogynous organization of the colony. The presence of two or more adult queens is possible only during the period of sociotomy when the separation of the new colony resulting in swarming is delayed due to unfavorable weather. The participation of two queens in carrying out the reproductive function is possible in colonies that replace the queens. However, their life together is usually not long.

Life expectancy and the reproductive potential of females. In social insects, females (female-foundresses) performing reproductive functions usually differ from their female offspring that appear in the initial phases of the development of the new colony. These females in social insects most often perform auxiliary functions and do not directly contribute to the reproduction of their species. This is most often associated with their sexual underdevelopment.

Female-foundresses. The lifespan of female-foundresses and their female offspring varies and depends on the level of the social organization of the bees. Female-foundresses of eu- and subsocial bees have a relatively low life expectancy. In species that have the lowest level of social organization, the female-foundress may not survive until her offspring reach the adult stage. Unequal trophic support at the larvae stage is the cause of differences in the development of reproductive organs. As a result, sexually mature females are engaged in the reproduction of offspring, and underdeveloped females perform subsidiary functions. This form of communality is known in the bees *Exoneura eremophila* and *E.*

setosa (Anthophoridae) settling in the core of the stems of herbaceous plants. Female-foundresses in these bees do not always survive until the appearance of the first underdeveloped females. When they reach the imago they remain in the nest, and after the emergence of mature females they participate in the protection of the nest and the trophic support of the generation of their sisters (Houston, 1977).

In higher-organized social species of *Ceratina*, for example, in *Ceratina japonica*, *C. flavipes* and *C. iwatai*, the female-foundress' care of developing offspring helps to reduce mortality from approximately 50% to 3-19% (developing individuals can exist without female-foundresses in the event of their death). The strengthening of the relationship between female-foundresses and the offspring of Ceratina is sometimes expressed (in about 10% of cases) in joint overwintering, and afterward the female-foundresses start to perform reproductive functions (Sakagami, Maeta, 1977). The increase in the life expectancy of female-foundresses related to the increased interrelation with their offspring is even more pronounced in some species of the Halictus bee. For *Lasioglossum* (*Dialictus*) *umbripenne*, the female-foundress lives for about a year, with the first generation of her offspring performing the function of working individuals. During this time, she is not replaced by her daughters (Wille, Orozco, 1970).

In one-year-old colonies of bumblebees, working individuals always develop in the first brood cell constructed by the female-foundress; their number in *B. agrorum* and *B. humilis* reaches eight (Alford, 1970), and in *B. hortorum* – 16 (Alford, 1971). In the next generations, mature females develop. The reproduction of males takes place at the end of the development season of bumblebee colonies. The lifespan of female-foundresses is limited to one year; workers and males die when the cold comes. Female-foundresses live much longer in perennial colonies inhabiting the tropics. However, in these colonies, several females of different ages may simultaneously oviposition fertilized eggs (Sakagami, Zuchi, 1965; Michener, Amir, 1977).

The activity of the reproductive function in bumblebee female-foundresses increases in the process of the development of their colonies. In the first brood cell, the female *B. agrorum* usually ovipositions eight eggs within three days. This is followed by a reproductive pause that ends after the emergence of the first working individuals, and after that new brood cells are built in the nest (Alford, 1970). With the increase of the number of adults, the reproductive activity of the female can reach 12 eggs per day (Brian, 1951, 1952). The reproductive potential of perennial tropical colonies is much higher than that of annuals. Within a day, the female *B. transversalis* can oviposition up to 300 eggs (Michener, 1979).

The female (queen) bees of the genus *Apis* are distinguished by their high fertility and lifespan. In different races of the honey bee, the queen can oviposition from a few hundred to three thousand eggs during the day, which, along with genetic differences, depends on the ecological situation and the number of working individuals in the colonies (Taber, 1980; Eskov, 1995a). The reproductive activity of the queens decreases with their age. The life expectancy of queens is generally limited to 4–6 years, but the maximum, according to the observations of A.D. Betts (Eskov, 2013), can be eight years. The death of the queens most often happens during the wintering period. The probability of death increases with age.

Worker individuals. Regardless of the level of social organization in bees, the underdeveloped females as a rule act as workers. They appear in seasonal colonies at the initial stages of their development. In social Halictus types, workers live for about 30 days (Wille, Orazco, 1979). In some types of Halictus, small underdeveloped females appear along with large, mature females in the first reproduction. They replace the female-foundress in the case of her death. In the colonies of *Exoneurella eremophia* and *E. setosa*, the representation of mature females can reach 75%. They perform a reproductive function with the female-foundress in the colony (Houston, 1977).

Males are usually developed from eggs ovipositioned by unfertilized worker bees. In some species, the contribution of working individuals to the reproduction of males is of crucial importance. Thus, in the colonies of bumblebees, the proportion of males developing from eggs ovipositioned by working individuals can reach 90%, and in the *Trigona* – 95% (Lin, Michener, 1972). The behavior of working individuals ovipositioning eggs differs from the behavior of female-foundresses. Workers are characterized by randomly ovipositioning several eggs in one brood cell. In this process, workers often occupy other cells, eat eggs and replace them with their own (Katayama, 1973). Females are obligatorily similarly related to brood cells constructed and occupied with eggs ovipositioned by working individuals (Garofalo, 1978).

The life expectancy of bumblebee workers depends on the forms of participation in the life of the colony. The bumblebees engaged in food delivery usually do not live as long as workers performing intra-nest work. In colonies of species inhabiting temperate climates, the average life expectancy of workers is limited to about 25 days, and the maximum lifespan reaches 69 days (Brian, 1952). This is similar to tropical species of *B. morio*, where foragers live on average for 36 days, but individuals who specialize in performing intra-nest work live twice as long – 73 days (Garofalo, 1978).

Among the bees of the genus *Apis*, working individuals begin to perform reproductive functions in the absence of the queen. In a queenless colony of a honey bee, up to 25% of bees can be found ovulating (Perepelova, 1928), while representatives of *Megapis* have only one (Velthuis et al., 1971). The number of eggs ovipositioned by the ovulating working individual in *A. mellifera* varies from 19 to 32 (Perepelova, 1928). The working bee spends 17–251 sec/s (Gaydak, 1969) ovipositioning eggs, and the queen spends about 10 sec. Convergent similarities of working bees and bumblebees engaged in ovipositioning eggs are expressed in disordered (chaotic) behavior.

The lifespan of honey bee workers is subject to seasonal variability. Bees actively participating in the replenishment of food reserves of spring-summer generations live for 30–40 days, and in wintering ones – up to 6–7 months (Eskov, 1995a). The maximum life expectancy of bees of spring-summer generations can reach 89 days, and for wintering – 304 days. Some bees can survive for up to 307–396 days in queenless colonies (Maurizio, 1958). The concentration of carbohydrate food consumed by worker bees has a significant effect on their life expectancy. An increase in water content leads to a reduction in life expectancy (Eskov, 1992, 1995a).

Determination of gender. Bees' gender differentiation is mainly associated with a cyclic replacement of haploidy and diploidy. Females in most known species of bees develop from fertilized eggs and males from unfertilized eggs. If you do not take into account the mutation process, then the kinship coefficient between the female-foundress and her daughters in a case of single pairing approaches 1:4, and in the case of a pairing with two males (polyandry), it increases up to 1:2; with a triple pairing – up to 5:12; and with a tenfold pairing – up to 3:10.

The probability of females mating with one or more males is related to species-specific features but may depend on the environmental situation. Polyandry is not widely spread among bumblebees. In most of their species, females mate with males at the end of summer and the beginning of fall. However, *B. hypnorum* females may have a 2-3-fold pairing (Roseler, 1978). The honey bee is characterized by polyandry. Queens of these species can mate during the mating season with 17 drones under favorable weather conditions, otherwise they remain unfertilized.

In the case of arrhenotoky, common in groups of honey bees living on the Eurasian continent, colonies with unfertilized queens are eliminated. The greatest probability of survival without fertilized queens is in the colonies of species characterized by thelytoky. This was first discovered in the bees of *A. mellifera capensis* inhabiting the Cape region of South

Africa (Onions, 1912). The colonies of these bees usually contain about 20% of individuals with enlarged ovarioles. Among these initially working bees, there are individuals that for 28–42 days oviposition unfertilized eggs, from which females hatch and develop. In Cape bees, the thelytoky is stimulated by the loss of the ovulating female. If there is a thelytoky in the colony, the drones will develop from eggs ovipositioned by working individuals. These bees are freely interbred with European bee races. When crossing *A. mellifera capensis* with *A. m. carnica*, the thelytoky dominates in metis, and in the case of crossing with *A. m. ligustica* this feature turns into a recessive state (Ruttner, 1977).

The development of drones from fertilized eggs has been discovered in honey and Indian bees. Bees usually destroy such drones in the early stages of ontogeny. However, sometimes they develop up to the imago stage and can occupy up to 30% of the places in the cells used for working individuals' reproduction (Woyke, 1980). Adult diploid drones differ in appearance from haploid ones, having similarities with females and males at the same time (mosaic gynandromorphism). The increase in the size of certain parts of the body can be due to polyploidy (Woyke, 1980). Gynandromorphism in diploid Melipona drones is expressed by the presence of similarities with females in the structure of their exoskeleton, wings, and eyes (Kerr, 1974).

The seasonal sequence of the reproduction of sexually mature females and males. In one-year-old bee colonies, the sequence of the reproduction of mature females and males, their time of flight from the nest and mating is determined by the hereditary program acquired in the process of adaptation to typical living conditions. For example, *Osmia lignaria* females complete development and leave the nest before males, whose size decreases from the first to the last generation. This is caused by a deterioration in trophic support (Torchio, Tepedino, 1980). In bees of *Lasioglossum (Dialictus) umbripenne*, reproductive periods of sexually mature offspring are associated with cycles of drought, but the seasonal cycle of the development of colonies is completed by the reproduction of drones (Wille, Orozco, 1970). During the second half of the summer, the reproduction of sexually mature offspring takes place in colonies of bumblebees living in temperate climate zones. In colonies of tropical species, the reproduction of males and females continues uninterrupted throughout the year. The female can live for more than one year. Nevertheless, new colonies are always based on the same female (Michener, Amir, 1977).

In colonies of the subsocial species where the females are completely incapable of independent existence, the cyclicity of the rearing of sexually mature offspring is usually associated with certain phases of the colony's development. For example, in a young colony of *Trigona nebulatakomiensis*, which contains about 200 individuals at the beginning of the development of a new nest, males are usually absent. Males appear in colonies in periods of time when the number of individuals increases. In colonies including 1-2 thousand adults, the representation of males may reach 7% (Roger, 1969). In colonies of the honey bee at the beginning of the active season, drones appear firstly among the sexually mature individuals, and only then among queens. The reproduction of drones is characteristic of all or most of the overwintered bee colonies, and the queens are reproduced in small numbers only by those colonies where bees are preparing for sociotomy. In bee colonies that have not found a queen, the reproduction of queens is possible at any time of the annual cycle if there are developing larvae of working bees in the nest.

Polymorphism and polyethism. The development of functional differentiation (polyethism) in bee colonies is associated with an intensification of morphophysiological differentiations (polymorphism) and the acquisition of functional specialization. However, functionally significant changes in species with a high level of social development occurred only among females. They diverged in working individuals and ovulating females (queens).

Females. In the eu- and subsocial species, female-foundresses and their assistants almost or completely do not differ from each other in appearance. There are no differences in the seasonal generations of females of Australian Halictus of the genus *Lasiglossum*, although they have developed collective nest protection and mutual construction activities (Knerer, Schwarz, 1976). The ovulating females of the genus *Evylaeus* practically do not differ in size from the individuals performing the functions of workers (Knerer, 1980).

A pronounced differentiation of females according to morphometric features, body weight and nest behavior can be traced in *Eulaemanigrita*. In these bees, small females remain in the nest and perform the function of working individuals while relatively large ones fly away (Zucchi et al., 1969). There is a similar situation in *Lasioglossum (Dialictus) umbripenne* (Wille, Orozco, 1970), *Exoneurella eremophila* and *E. setosa* (Houston, 1977), where the first generation of relatively small females participates in foraging, building and nest protection, although many of them (about 50%) have developed ovaries and can mate and oviposition eggs in the presence of the female-foundress in the nest. Small females rarely participate in ovipositioning.

The reproduction of physiologically underdeveloped workers at the initial phases of the development of colonies is mainly due to a lack of trophic support in the larval stage. In most biological situations, underdeveloped females cannot mate, but this does not deprive them of the opportunity to assist the female-foundress in the reproduction of sexually mature offspring. The ratio of adult workers and developing individuals influences the reproduction in the colony of sexually mature and underdeveloped females. Their representation decreases with an increase in the number of adults participating in the trophic supply of larvae. That can be clearly seen, for example, in annual bumblebee colonies (Puovrean, 1971).

The sequence of transitional forms reproduced in annual colonies is a result of the differentiated supply of food to the larvae from the beginning to the completion of their development. However, their functional differentiation may not have a strict connection with the size of individuals. Regardless of the size in *B. (Fevidobombus) morio*, the specialization is expressed by the fact that one part of the individuals is engaged in foraging, and the other performs intra-nest work. Depending on the biological situation, some females can participate with them in the delivery of food, heating the brood, and guarding the nest (Garofalo, 1978). In *B. agrorum*, large bumblebees usually bring pollen and nectar into the nest, and small ones – only nectar (Brian, 1952).

Among eusocial species, the presence of transitional forms between the largest queens (Darchen, Darchen-Delage, 1974) and working individuals is widespread in stingless bees. They have the size of working individuals that increases, accordingly, with the growth of their quantities in colonies, which is determined by the improvement of trophic brood breeding (Imperatriz-Fonseca, 1976). Along with the size of the body, the queen differs from the working individuals in a variety of morphophysiological features. The worker individuals are stronger in the eyes and mandibles. The specific features of worker bees include the presence of a specialized device for collecting and transporting pollen on their third pair of legs. The queen differs from the working individuals in its rather developed ovaries as well as the structure of the abdominal nervous system. In the queen, from the fourth to the seventh segment of the abdomen, ganglia are displaced to the anterior part of the body, and in the workers – to the rear (Darchen, Darchen-Delage, 1975).

The queen and the workers of the *Apis* bees differ in a large set of characteristics. The phenotype of females at the stage of the larva is modified by their diet. The larvae of workers in *A. mellifera* are supplied with royal jelly (the secret of the hypopharyngeal and mandibular glands of adult bees) only during the first two days, and the queens – until the end of the larval stage. As a result, the queen at the beginning of the imaginal stage is approximately twice as big by mass than the working individuals. The queen differs from working bees in the absence of wax glands and morphological structures for collecting pollen, and the eyes and the oral apparatus are less developed, but the reproductive organs are hypertrophied. European groups of honey bees have about 200 ovarioles in the ovary of the queens, workers of *A. mellifera ligustica* have on average 12 (Chaud-Netto, Bueno, 1979), and *A.m. capensis* – 19.6 (Velthvis et al., 1971).

The presence of an underdeveloped reproductive system in worker bees allows them, under certain conditions, to participate in the reproduction of males (*A. m. mellifera*) or females (*A. m. capensis*). Ovulating working individuals in breaks between ovipostioning can engage in the construction of honeycombs, feeding larvae, foraging and other intra- and out-of-nest work.

Polyethism in colonies of eusocial species is not associated with strict morphophysiological differentiation. The functions usually change with age. Among the bees of spring-summer generations, young workers aged between 3 and 12 days are usually involved in brood rearing. They have actively functioning hypopharyngeal glands at this time. After 11-13 days, the development of wax glands in bees is intensified, which allows them to actively participate in the construction of honeycombs. After 17–21 days, bees start being engaged in food delivery. Young foragers can participate in the protection of the home, localizing at the entrance of the flying hole.

The marked age differentiation of the performed function can be modified under the influence of various extreme factors. For example, in a colony consisting only of young bees, their participation in foraging can begin at an age of 5-8 days old. In contrast, the bees of the fall generation, having lived for several months, participate in the brood rearing. So, the age dependence of polyethism is observed only in the presence of bees of different ages in the colony.

Males. Unlike females, males do not participate in the life support of their colonies. In seasonal colonies, they often leave their nests after reaching imago and puberty. The assumption of the presence in the colonies of some Australian Halictus *Lasioglossum (Chilalictus) sp.* males involved in the protection of the nest (Houston, 1970) was not confirmed (Knerer, Schwarz, 1978). These males, distinguished by the presence of an enlarged head and mandibles, resemble representatives of termites. The emergence of such males in the Halictus genus is caused by a lack of trophic support in the larval stage. These males do not leave their nests because their wings and vision are underdeveloped (Knerer, Schwarz, 1976). Perhaps drones in the nests of Melipona *Schwarziana quadripunctata* can take part in the processing of nectar; this occurs during its consumption (Impratriz-Fonseca, 1976).

The morphophysiological and morphometric variability of drones is mainly associated with their trophic supply. The size of the cells is important here. For example, in Halictus, the enlargement of drones occurs in the case of the development of cells for the reproduction of females, which are abundantly supplied with food (Knerer, Scwarz, 1978). In Melipona, the size of drones developing in queens' cells increases by approximately one-third (Inperatriz-Fonseca, 1976). In the honey bee, drones usually develop in enlarged cells. However, in some biological situations, drones sometimes develop in relatively small cells of a working type that leads to their underdevelopment – a 1.8-fold decrease in body weight (Eskov, 1995a).

In the nests of the honey bee, young drones come into tactile contact with working individuals during the first 4-5 days of the imaginal stage and receive food regurgitated from the crop from them. With age, the attractiveness of drones decreases for worker bees. Being in need of care, young drones are usually located in the center of the nest, and the older ones are on its periphery. The first flights of drones from the hive start at the age of 4-8 to 14 days, and mass flights start during the mating season from the age of 16 days (Muszynska, 1980). In bees inhabiting cold and temperate climate zones, the expulsion of drones from the nest occurs before the beginning of wintering.

Mechanisms of Social Consolidation

The dominance of ovulating females. The signs of dominance or pronounced mutual aggression are usually not observed in the colonial populations of bees, although each ovulating female mainly cares about her offspring. Conflicts are possible only when capturing others' cells; this is often accompanied by the removal of other females' offspring from them. Competition for nesting sites becomes acuter with an increase in the population density of the colony. In the small communal nests of the Halictus *Conanthalictus dicksoni* and *C. conanthi,* one nest may be occupied simultaneously by 2-3 females similar in appearance (Rosen, Meginley, 1976). Similarly, polymorphism and polyethism were not discovered in the ovulating females of the *Lasioglossum sp*., which were co-participants in the settling of their settlements (Knerer, Schwars, 1976).

In colonies of social types, females usually differentiate into dominant and subordinate. Dominant females are mainly engaged in the reproduction of males and other females, and subordinates are engaged in the trophic support of the colony, construction, and the protection of the nest. The means used by females to achieve a dominant position can be conditionally divided into ethological and physiological ones.

Ethological domination. The hierarchy of females in bee colonies evidently developed from the inequality of their trophic support in the larval stage. In eu- and subsocial one-year colonies of bees, the first generations of females are not adequately provided with food, which is reflected in their underdevelopment. Comparatively to the female-foundress, they are usually less in mass and poorer in the development of the reproductive organs. This is due to the fulfillment of the auxiliary work in the nest, although working individuals are capable of ovulation and often participate in the reproduction of males.

Possessing an advantage in strength, female-foundresses and/or sexually mature females counteract the reproduction of offspring by underdeveloped females and working individuals. Large mature females, competing in the struggle for reproduction with underdeveloped females, destroy the eggs ovipositioned by them and use the freed cells to reproduce their own offspring. This limits their contribution to the population of underdeveloped females.

Among bees with different levels of social organization, the practice of dominant females eating eggs ovipositioned by working individuals is widespread. This behavior is known in the colonies of the primitive Halictus *Lasioglossum zephyrum* (Michener, Brothers, 1974), in more socially organized advanced bumblebees (Garofalo, 1978) and in eusocial species of bees. In Melipona, the process of eating eggs ovipositioned by working individuals by sexually mature ovulating females (queens) has become one of the acts of the realization of the reproductive instinct. The eggs of workers began to be used by the queens as a trophic substrate, which compensates for the costs of ovulation.

Another form of cannibalism developed in colonies of bees of the genus *Apis*. The number and age of larvae eaten by worker bees mostly depend on the productivity of the feed area used by bee colonies and their physiological status. Indian bees feed up to about 95% of the brood before sealing during periods of abundant planting of nectar and the production of pollen. During moderate productivity of the food area, the number of larvae that survive to the pupa stage decreases to 50%, and while fasting the reproduction of the working individuals is suspended, although the queen ovipositions eggs (Woyke, 1976).

The activity of cannibalism by honey bees is subject to seasonal variability. 75–80% of bees develop from the egg stage to the imago in spring, up to 80–90% in summer, and 50–75% in fall. Most workers usually destroy developing drones. The exception is colonies that have lost their queens. In such colonies, even in fall, the consumption of developing drones is about 25% (Woyke, 1980).

The eating of brood by Indian honey bees has no direct relation to the dominance of the queens. However, this is associated with maintaining the homeostasis of the colony and regulating its development in accordance with the environmental situation. The regulative role of cannibalism is expressed in the fact that worker bees, while eating part of the brood, provide an improved trophic supply to the remaining larvae and queen. In contrast, in the one-year-old dominance colonies, the female-foundress can delay the reproduction of males for a time and increase the reproduction of females.

Tactile contacts and trophallaxis. Singly living species of bees tend to have an indifferent attitude towards their offspring. The offspring of the female-foundress also often do not contact her or each other. The origin of sociality is associated with the acquisition of the caring instinct for developing offspring by the female-foundress, which can be expressed in its protection. The strengthening of the connection of the female-foundress with her offspring is expressed in the periodic supply of larvae with food. The primitive methods of feeding larvae include regurgitating the contents of crops on them, which occurs in certain species of Ceratina that use common brood cells for offspring placing (Sakagami, 1960). The Ceratinas *C. japonica* and *C. flavipes*, nestling in the stems of plants, are forced to destroy the barriers between the sealed cells to feed the larvae. The beginning of the defecation of larvae probably gives a signal to females. The female, after unsealing the cell, removes the excrement and then seals it again (Sakagami, Maeta, 1977).

The tactile interaction of female bumblebees with developing offspring often precedes the delivery of food to the brood cells. This is expressed by the fact that the adult individuals firstly sense the larvae with antennae and only then supply it with food (Sakagami, 1976; Sakagami, Zucchi, 1965). However, this basically limits tactile interaction to the bumblebee colony. Specific forms of interaction of adult individuals are known in *B. agrorum*, where foragers that return to the nest can attack the passive individuals in it. Sometimes bumblebees in the nest attack foragers returning to the nest and lick the nectar regurgitated by them (Brian, 1952). Bumblebees usually consume pollen from the feed cells and very seldom take it directly from the feet (from the pollen baskets) of foragers (Sakagami, Zucchi, 1965).

By means of trophallaxis, female-foundresses of some species of carpenter bees living in South Africa interact with their offspring (Watmough, 1974; Bonelli, 1977). The female-foundress of *Xylocopa combusta* often enters into trophic contact with young adult individuals (Watmough, 1974). In the *Lasioglossum zephyrus,* returning foragers firstly contact the female-foundress, and then release their burden from their fodder. Their tactile interaction is usually maintained in the process of progressing to the feeding cell (Breed, Gamboa, 1977).

The trophallaxis and tactile interaction in the colonies of bees of the genus *Apis* reached a high order level and perfection. Their isolated group of workers exchange food with each other. The frequency of trophic contacts increases with the number of bees in the group. By the radioactive labeling of feed obtained by a solitary-living bee, it was established that it can be distributed in a group of 20 bees in 6 hours (Skirkavichyus, Buda, 1975). Food and other substances are distributed among different polyethical groups in the nest of the bee colony even faster, but at different rates. The greatest activity of food exchange is in bee-foragers and the smallest of those that perform intra-nesting work (Nixon, Ribbands, 1952).

The amount of feed distributed during trophic contacts depends on the individual characteristics of the donor bees. In total, about 7% of bees transmit up to 90% of the contents of crops in the process of trophallaxis, about 50% – from 50 to 60%, and about 40% – up to 5% (Duo et al., 1975). The majority of the contents of crops (an average of 60%) is transferred to queens (Pen et al., 1975).

Not all drones always receive food from worker bees. In the active period of the colony's life, approximately 10% of drones consume food from honey cells. In the process of trophallaxis with worker bees, about 50% of drones satisfy their nutritional needs. In the process of trophic contact, the bees can give the drones up to 95% of the contents of the crop (Duo et al., 1975).

In the process of the trophic and tactile contacts between members of the bee colony, pheromones that fulfill the regulatory function are distributed. At present, about 30 varieties of different pheromones are found in the honey bee, differing in the mechanisms and results of their physiological effects on the organisms of adults and developing bees. Royal jelly, which is distributed by the bees in contact with the queen, has especially high activity and a wide spectrum of action. After coming into contact with it, the bees become activated and within minutes enter into trophic or tactile contact with other bees (Ferguson, Free, 1980; Seeley, 1979). The initiators of contacts are usually bees who left the surroundings of the queen (Butler, 1980; Skirkyavichyus, 1986).

Conclusions

In the development of the strategy of nesting behavior, an important role belongs to the acquisition of the instinct responsible for offspring reproduction in individual cells. The possibility of differentiated food supply and regulation of the diet at the larval stage is associated with developing individuals. This excludes intra-species competition between developing individuals. Their morphophysiological differentiation is mainly due to the inequality of trophic support, which can vary in weight and chemical composition.

In the process of the development of building instincts, selection favored the preservation and improvement of those that ensured an increase in independence from the unfavorable factors of the external environment. On this basis, eu- and subsocial species settling in the soil developed devices for constructing the waterproofing of brood cells, thus protecting the feed from waterlogging or drying. Solitary living and social species of bees have adapted to use resinous plant extracts as a waterproofing material. Increasing their independence from the habitat was achieved by the development of morphophysiological adaptations which allow them to use their own secretions for waterproofing and/or building cells, among which waxes and wax-like substances have become widespread. In species that do not have specialized wax excretory organs, the use of epicuticle waxes has been obtained. The improvement of wax-using mechanisms is associated with the development of waxing glands.

The process of the order of the nest's structure was motivated by the need to enlarge the nest's size, which was associated with the increase in the number of members of the colony. In primitive social species with a relatively small number of bees, the brood cells do not have a strict ordering. A special disorder to the nesting design is typical for different types of bumblebees. They, like stingless bees, re-use the building material, which contributes to reducing energy costs for nest structures. However, this practice has not developed in the honey bee, which reached the highest level of social organization.

In a honey bee nest, honeycomb cells do not have a strict differentiation between food stores and brood cells. This provides the mobility to use the limited nesting space for the reproduction of working individuals and drones in the spring and summer periods, and for storing food in the freed brood cells. After the completion of the brood's reproduction, food is placed in the cells that occupy the most part of the honeycombs, which is necessary for wintering bees. They do not feel the need to migrate in search of food when they aggregate under the influence of cooling in a limited part of the nest (in the food cells). Therefore, with the acquisition of the instinct of settlement in shelters, the construction of vertical two-sided honeycombs and the use of cells for the reproduction of brood and/or the storage of reserves at different times of the annual life cycle is related to the development of zones with a temperate and cold climate.

Social relations in bees are based on the development of the caring instinct for the offspring of the female-foundress and her first generations, which are physiologically underdeveloped females. The underdevelopment of females, causing their partial or complete elimination from reproduction, in eu- and subsocial species mainly occurs in connection with the restriction of the trophic supply of larvae, which can have a cyclic character under the influence of seasonal variability in the productivity of the food place. Underfeeding is their limited direct contribution to the population, to which the first generation of females is subjected. Nevertheless, they contribute to the female-foundress in the reproduction of her offspring, which, thanks to the abundant trophic support, reaches normal sexual development.

The acquisition and improvement of the means of dominating by the female-foundress, which contributes to the consolidation of colony members, is associated with a decrease in the contribution to the population by its potential competitors, referred to as assistants. A widespread method of domination, based on eating the female-foundress' eggs that are ovipositioned by underdeveloped females, eliminates them from reproduction. Using this method, the female-foundress or another mature female replenishes the reproduction costs. The physiological dominance that reaches its highest perfection in eusocial species is associated with the development of trophallaxis and tactile contacts. They ensure the distribution of pheromones, thereby increasing the interconnectivity and interdependence among members of the bee colony.

Colony development in bees is associated with increased interconnectivity and interdependence between adults and developing individuals. Their progressive consolidation, which reaches its highest perfection in eusocial species, has facilitated the transformation of the colony into a biological unit that is individually subject to the actions of natural selection and other factors of evolution. The genetic relationship of the offspring of ovulating females and its development under similar conditions, excluding intra-breeding competition, caused the emergence of colony selection. It involves the acquisition of a specific form of reproduction by means of sociotomy, which provides an increase in the number and expansion of the range occupied by eusocial species. With high life expectancy, which is characteristic for queens, in the process of sociotomy (Darchen, Delag-Darchen; 1975; Eskov, 1992), the replacement and renewal of genotypes of bee colonies occur.

CHAPTER TWO

TEMPERATURE ADAPTATIONS

The honey bee is present in a wide area, including vast territories on all five continents – from the tropics to the subarctic. This is due to the acquisition of aneurythermality – the ability to survive in a wide range of temperature changes.

The ancestral form of the genus *Apis* is not established. It may have formed among the bees of the genus *Electrapis Cock.*, 1908, which became extinct in the late Eocene. Compared to the known fossil forms, these bees resemble *Apis* in their complex of morphological characters, but they are similar to bumblebees (*Bombus*) in their signs of venation. The most ancient representatives of the genus *Apis* are fossil species: *A. (Synapis) henshaw*; *Cock.*, 1907 and *A. (Synapis) cuenot* (Theobald, 1937; Buttel-Reepin, 1903; Ruttner, 1988; Radchenko, Pesenko, 1994; Rasnicin, 1980).

Fossil representatives of *A. mellifera* are relatively young. They were found in East Africa in copal (fossil resin) of the Pleistocene (Petrov, 1992; Buttel-Reepin, 1903). This era of the Quaternary period was characterized by a general cooling of the Earth's climate. Obviously, the cooling affected the development of the cold adaptations complex in *A. mellifera*, which ensured the survival of the species in temperate and cold climates. Along with their ethological and physiological adaptations, bees have also adapted to their settlement in the shelters used as a house.

Bees' houses

Under natural conditions, honey bee colonies settle in various shelters that include hollows of trees, crevices of rocks or voids in the ground. Shelters are necessary for bee colonies to protect themselves against adverse weather conditions and biotic factors. The internal cavity of the shelter is used to attach the cells where feed stocks and developing individuals are placed. All artificial structures for keeping bees have the same purpose.

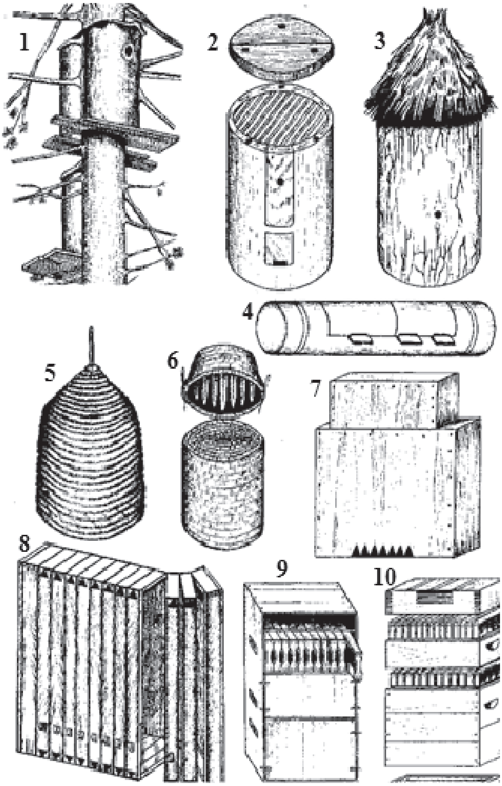

Fig. 6. Structures for beekeeping: 1 – Hole to a hive in a tree, 2–4 – packs, 5–6 – straw hives, 7 – wooden hive with an extension, 8 – Guber's book hive, 9 – Prokopovich's hive, 10 – Danan-Blatt's hive.

With the development of beekeeping, the design of hives was modified in accordance with the improvement of the technology of keeping bees (Fig. 6). Beehives are made of various natural and synthetic materials. At the same time, the main structural elements of the hives have not undergone fundamental changes since 1814 when P.I. Prokopovich designed the folding frame hive, and L.L. Langstroth formulated the basic principles of the hive system of keeping bees (Langstroth, 1909).

The natural colonization of dwellings by bee colonies occurs in the process of sociotomy (breeding by dividing maternal colonies) or relocation. When choosing a new home, bees evaluate its physical properties. In the forest zone, bees prefer to settle in the hollows of trees with a volume of 30–60 thousand cm^3 (Seeley, Morse, 1976). In the presence of hollows, the bees settle mainly in those whose volume is in the range of $60–80 \cdot 10^3$ cm^3 (Petrov, 1983).

The settlement of a new home is accompanied by an increase in its temperature. In an empty hive, a colony containing about 25 thousand bees increases the temperature from 19 to 37 °C within 15–20 minutes. Subsequently, during the period of mastering the dwelling, the temperature decreases in it and is maintained at a relatively high level – within the limits of 33-36 °C – only in the area of building honeycombs.

In the initial phases of the home's development, the distribution of bees in it depends on the location of the queen. Around her, there is an aggregation of bees and the initial formation of a thermal center. A high temperature is necessary for the bees to secrete wax, a large amount of which is required for the construction of nesting structures.

The honey bees' nest buildings are wax cells. The composition of beeswax includes esters, hydrocarbons, organic acids, various alcohols, resins, vegetable pigments, mineral and aromatic substances (about 300 different substances in total). The thermal conductivity coefficient of wax ranges from 3.47 to $8.16 \cdot 10^2$ W/m^2.

Honeycomb cells are used for the development of worker bees and drones from egg to imago. The diameter of the cells used for the development of worker bees varies from 5.00 to 5.55 mm, and the depth – from 10 to 12 mm. The thickness of the honeycomb with unsealed brood averages 22 mm, and after sealing it increases to 25 mm. The volume of a fully built cell is about 280 mm^3. The reuse of honeycombs for a growing brood leads to a decrease in the volume of cells as a result of layering cocoons (Mikhailov, 1927). This stimulates the bees to gnaw out of the cocoons and complete the walls of the cells, increasing their depth.

The cells used for the development of drones differ from bees' ones in size. Their diameter averages 7 mm, and their depth – 13-16 mm. Like bee cells, the drone ones are used to store carbohydrate foods.

For the development of queens, bees build specific cells – queen cells. The construction of queen cells occurs during the preparation of colonies for reproduction, or with the substitution or death of the queen. Queen cells rebuilt during the breeding period of the colony are located mainly on the periphery of the cells.

In terms of size, queen cells are the largest cells among the nesting structures of bees. For queen cells, an acorn-like form is typical. Their length rarely goes beyond 20–25 mm. Unlike bee and drone cells, queen cells are never used for reproducing queens, nor for storing food.

Regardless of the house design, the natural bee's nest forms a system of honeycombs arranged in a vertical plane. Their number and size are limited by the volume of the inner plane of the dwelling. This influences the shape of the honeycomb. Thus, nests built in hollows of trees form a honeycomb attached to the ceiling and side walls. Honeycombs located on the side walls usually do not have a solid attachment. Bees use undeveloped areas of the honeycomb as passages. When settling into narrow cavities, bees build one or several cells parallel to the walls.

Natural nests do not have strict orderliness by the cells' location (Ruth, 1938; Seeley, Morse, 1976; Petrov, 1983; Eskov, 1990, 1992). In the hollow or side, the cells form a complex system of labyrinths. In different zones, the cell can significantly vary in size and shape. Their thickness in the upper part of the nest (near the ceiling) can reach 45–50 mm, and at the bottom it decreases to 2–3 mm.

Usually, the bees begin the construction of several honeycombs in different parts of the nest cavity, attaching them to various projections. The localization of a bee entrance has some influence on the orientation of the cells. Most often, the cells constructed near the entrance are oriented along its longitudinal axis or at a small angle to it.

When using the hive system for keeping bees, the program for the construction activity of the bees is defined by the shape and placement of the framework with an artificial wax. It does not contradict the biological needs of bees. The size and shape of cells are not significant for them. But what is of fundamental importance is the observance of the biologically important parameters of the bees' nest in the hive. These include the parallel placement of cells in the vertical plane and the distance between them. Inter-cell space is usually maintained between 8 and 13 mm.

There are some racial differences in the sealing of feed cells. In particular, the European dark bee and the Krajina and Carpathian bees, when sealing the cells filled with honey, leave an air gap under the cap. Gray mountain Caucasian and yellow Caucasian bees do not leave an air gap between the honey and the cap. Italian bees have an intermediate position on this trait.

The genotype is associated with the potential productivity of wax production. In some hybrids, it reaches a very high level. This is expressed in the construction of the honeycomb of the entire free space of the hive and the construction of many wax walls. In contrast, inbreeding lowers the secretion of wax. Inbred colonies do not seal mature honey for a long time, and only use the foundation as the basis for honeycomb construction slightly or not at all (Moeller, 1976).

Under natural conditions, working bees and drones occupy the entire cell by the end of the larval stage, taking a horizontal position or a position close to it. A slight deviation of the longitudinal axis of the body of the older larvae, the prepupae and the pupae from the horizontal (usually upwards) corresponds to the inclination of the cells. In this position, the bees and drones spend the prepupal and the pupal stage. Developing queens also unwarp before pupation, but are positioned head down (Fig. 7). The axis of the queen’s pupa body in the queen cell, located on the lower part of the honeycomb, forms a right angle with the horizontal. The developing queens in the queen cells are localized on the sides of the cells with a small deviation from the vertical.

The orientation of the honeycombs can sometimes deviate from the norm, for instance, when the dwelling's position changes (overturning of the hive or if the tree in the hollow of which the bees live falls down). Local damage to nesting structures is also possible, for example, due to the fact that the cells break off due to a weak attachment to the inner surface of the dwelling.

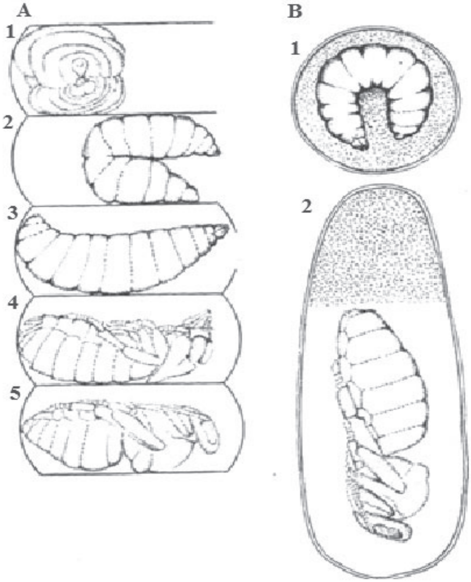

Fig. 7. Localization of the developing worker bee and the queen in the cells: A – the worker bee (1–3 – changes in the position of the larva before the cell sealing, 4–5 – the pupae in the sealed cells); B – the queen (1 – the larva stage, 2 – the pupa of an older age).

The orientation of the cells relative to the gravity vector is programmed in the nest-building instinct. A number of cells with larvae of working individuals or drones turned upside down by open sides are completed by bees, who deviate them from the vertical by 40–50°. When the rebuilt cells are turned, bees do not change their inclination but finish some of the wall building, adding 1–3 mm.

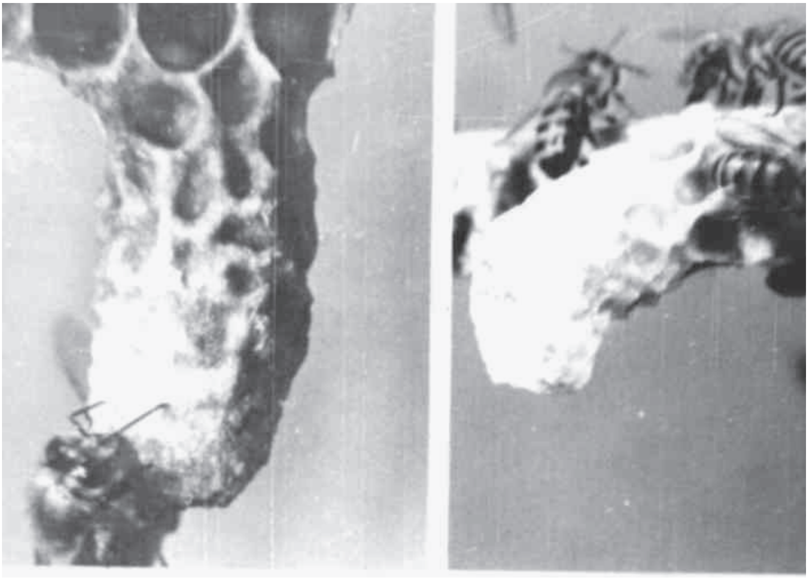

Fig. 8. Queen cell: rebuilt in a normal position (left) and rotated at the larval stage of the queen's development by 90° relative to the gravity vector (right).

The form of queen cells deviating from the natural position relative to the gravity vector is significantly modified. Their considerable reorganization occurs before sealing. Queen cells, rotated by 90°, are sometimes completed almost at right angles to the base (Fig. 8). However, most often, the downward deflection is a smooth bend.

Bees bend queen cells that are turned by 180° at the beginning of construction, with the presence of young larvae in them, first to the side and then upwards. Bees tend to seal queen cells with larvae of middle and older age that are 60-70% developed without a significant change to the angle of inclination. In the presence of a support (honeycomb, a neighboring queen cell, etc.), the bees often attach the top of the sealed queen head, facing up.

The angle of the queen cell's turn affects its size according to the completion of the larval stage of the developing queen. The length of the queen cells oriented downward (normal orientation) was 26.7 ± 2.3 mm on average, and upwards – 16.1 ± 1.2 mm. Queen cells were rotated 180° 1.9 ± 0.6 days before sealing.

The disorientation of the cells with larvae of bees and drones had a different effect on their viability depending on the stage of development. The reorientation of the cells with eggs by 90° (being placed in the upper-frame space) led to the elimination of 80–90% of individuals. At the same time, the death of 2–4-day-old larvae was 55 ± 16%. The larvae that survived to the prepupae stage were sealed by bees. But after 1–3 days, the bees opened the cells and removed the individuals from them. In the case of the incubation of brood sealed in a thermostat at 35 °C, 99.1 ± 0.7% of bees died in the cells turned upwards. They died mainly at the middle and older pupae stage. In the cells turned downward, most of the bees died at the stage of prepupae and pupae of a younger age. About 3% of the pupae reached the stage of adults who died in sealed cells.

A turn of 90° up or down from the horizontal of the cells with working bees or drones that were at the pupal stage did not affect the reduction of their viability. 99.6 ± 0.3% of the bees reached the imago stage and left their cells. This corresponded to the viability of the bees, which developed simultaneously in the vertical cells. Similarly, no effect on the viability of drone pupae was found by changing the orientation of honeycomb cells.

The impact of changes to the orientation of the queen cells on the viability of queens depends on the stage of their development. The bees rearrange the queen cells rotated by 90° or 180° while the queen is in the larval stage. This normalizes the development of queens and most of them reach the imago stage. But they all die if the queen cell is rotated 180° for 1.5–2.5 days before sealing. The death of most of them occurs at the stage of prepupae or pupae of a young age.

Temperature tolerance

The temperature adaptations of a honey bee clearly differ between individuals and are not directly connected with the development of eusociality and sociality. The acquisition of eusociality and sociality are associated with the transformation of the bee colony into a biological unit, which is individually subjected to the action of natural selection and other factors of microevolution (Eskov, 2003). Accordingly, individual adaptations maintain similarities with adaptations of singly living bee species and many other insects.

The mobility of the bees to respond to changes in ambient temperature depends on the sensitivity of the thermoreceptors. They are located on the distal segments of the antennae, but the phonoreceptors also react to temperature changes (Eskov, 1975).

Temperature receptors

According to their specificity of functioning, thermoreceptors are divided into thermal and cold types. The first of them react by increasing the frequency of impulses to heating, the second – to cooling. The bee reacts to changes in temperature by changing the frequency of impulses coming from thermoreceptors to the central parts of the nervous system.

The function of cold receptors in bees is performed by the whole-conical and ampule-shaped sensillae located on the 8 distal segments of the antennae. Sensillae react with a 10-fold increase in the frequency of impulses to the temperature decreasing by only 3 °C. The receptors quickly adapt to cooling, which results in a decrease in the pulse repetition rate by about 2 times within 1 s after the indicated decrease in temperature. The sensitivity of the receptors is refined to tenths of a degree.

The whole-conical sensillae are localized in the distal zones of the 8 antennal segments on their ventral and dorsal sides. The cuticle apparatus of a sensillum is represented by cone-shaped hairs with a length from 10 to 20 μm and a width at the base of about 10 μm. Each hair is placed in a mushroom-like cuticle capsule, the inlet of which coincides with the projection on the top of the hair. It falls approximately at the center of the widest part of the cuticular capsule.

The ampoule-like sensillae are very similar in appearance to the whole-conical sensillae. The main morphological differences are expressed in the fact that the ampoule and/or cone-shaped hair of the ampoule sensillae is immersed into the cuticular cavity more than that of the conical sensillae, with a smaller diameter of the inlet leading to the cuticular cavity. About 200 ampoule-like sensillae are localized on each antenna of a working bee (Lacher, 1967).

Specialized thermal receptors have not been found in bees. This function is combined with carbon dioxide receptors. They react by increasing the frequency of impulses both to heat and carbon dioxide concentration increases. Depending on their structure, the receptors are of the whole-conical and ampoule-like types (Lacher, 1964) or are whole-spherical sensillae (Yokohari, 1983).

Whole-spherical sensillae are localized among other types of sensillae predominantly in the middle part of the antennal segments. The worker bees and drones have about 50 such sensillae. Each of them is bordered by a plate-shaped depression. The cuticular apparatus is placed in its central part. Its distal part protrudes only slightly from the cavity formed by the puffin off of the cuticle. The diameter of the cuticular apparatus is about 3 microns, and the height – 4 microns. The gap between the cuticular apparatus and the cavity wall expands from 0.2 μm at the entrance to 0.5 μm as it approaches the base. The cuticular apparatus contains no input channels or pores. With an electron microscope, it was found that the central part of the cuticular apparatus is characterized by the highest electron density.

The sensitive apparatus contains 3 or 4 receptor cells. The inhibitory cell limits the external cavity of the receptor, adjoining the inner side to the trichogenic cell. Its membrane forms a cylindrical cavity through which processes of three sensitive cells pass. In the capsule of the cuticular apparatus, they form micro-branches. The nucleus of the braked, trichoid and sensitive cells are at approximately the same level in the proximal part of the receptor (Fig. 9).

Hyperthermia

Imago stage. *Lethal effects.* The tolerance to hyperthermia depends on the physiological state and ecological situation of the bee. The lethal effectiveness of heating for bees increases with an increase in external temperature (Eskov, 1998). In the case of 25 ± 5% relative air humidity, bees can live at 45 °C for 297 ± 36 min; at 50 °C, they can live for 107 ± 11 min; and at 55 °C, they can live for 31 ± 8 min. Drone bees are less tolerant to hyperthermia; middle-aged drone bees die in 72 ± 18 min at 50 °C (Eskov, Babkina, 1990).

An increase in air humidity elevates the lethal effectiveness of the temperature factor, which is associated with a decrease in heat loss from the bee’s body via water evaporation. The concentration of the carbohydrate feed in the honey bag has an opposite effect. Other conditions being equal, bees whose bags are filled with water are able to survive sublethal temperatures for the longest time. The decrease in the tolerance to hyperthermia associated with an increase in the concentration of the carbohydrate feed is determined by its effect on the heating of the bee’s body. The more calorifacient the feed, the higher the heating. Therefore, the lethal effectiveness of hyperthermia is increased by the activation of thermogenesis. Conversely, the presence of water in the bag intensifies cooling due to its evaporation through the body covers and respiratory system.

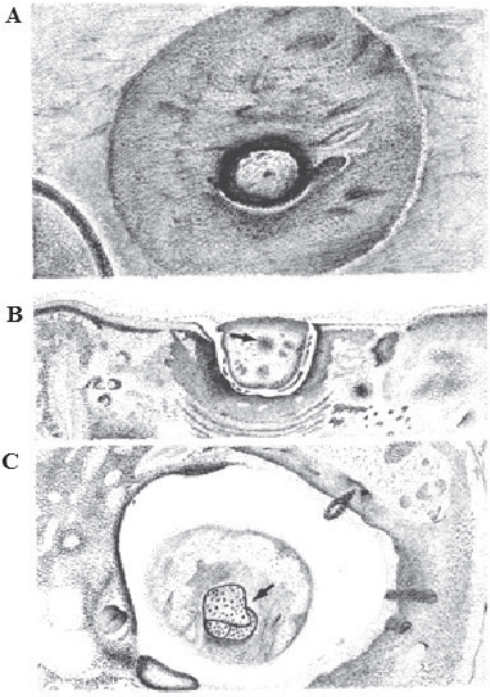

Fig. 9. The microstructure of the whole-spherical sensillae combining the function of carbon dioxide and temperature receptors: A – appearance (x 12,000); B – sagittal cuticle of the cuticular apparatus; the arrow shows its electron-dense part (x 16,000); C – cross-section through the middle part of the cuticular apparatus, indicated by the arrow, where the three processes of the sensitive cells are located (by: Yokohari, 1983).

The consumption rate of the carbohydrate feed contained in the honey bags depends on its water content. Independently of this, feed consumption increases when hyperthermal exposure approaches lethal values. For example, the 30% sucrose contained in the bags is consumed at a rate of 0.13 mg/min for the first 25 min at 50 °C and at a rate of 0.28 mg/min for the next 50 min; for 60% sucrose, the corresponding rates are 0.27 and 0.43 mg/min.

The local heat exposure of bees has different lethal effects depending on the heated body part. A bee survives heating to the front side of its head to 50 °C for 150 min (minimum, 44 min, and maximum, 252 min). Similar heating to the dorsal side of the abdomen ends in a lethal outcome 3–17 times faster (Eskov, 1995a).

Darkening of the body covers precedes the bee's death from hyperthermia; this is connected with the dissolution of epicuticular waxes. This process starts with the dissolution of wax on the abdominal surface. At 50 °C and 20–50% air humidity, the surface layer of wax commences dissolving after 15–44 min. Further hyperthermia leads to further dissolution of the wax, which causes the bee's body to darken, resembling its moistening. Most frequently, bees die immediately after this or after a certain period of time at an optimal temperature. If hyperthermia stops at the initial stages of the darkening of the bee's body, its body cover is restored to its initial appearance; however, the potential lifespan of the bee is reduced.

The tolerance of adult bees to hyperthermia increases from the beginning of the adult stage to the age of 7–8 days; however, with aging, this trend changes to the opposite (Fig. 10). Immediately after leaving their cells, bees are able to live at 50 °C for 85 ± 27 min; after 12 h, their lifespan on average increases 1.1-fold; after 3.5 days it increases 1.5-fold; and after 7.5 days it increases 1.7-fold, reaching 141 ± 17 min. By middle age, bees' tolerance to hyperthermia in spring-summer generations decreases slightly; this decrease continues with aging. As compared with 7.5-day-old bees, the lifespan of 14-day-old bees at 50 °C decreases 1.1-fold, while that of 21-day-old bees decreases 1.2-fold, that of 60-day-old bees decreases 1.9-fold, and that of 180-day-old (overwintered) bees decreases 2.3-fold (Eskov, 1995a, 2003).

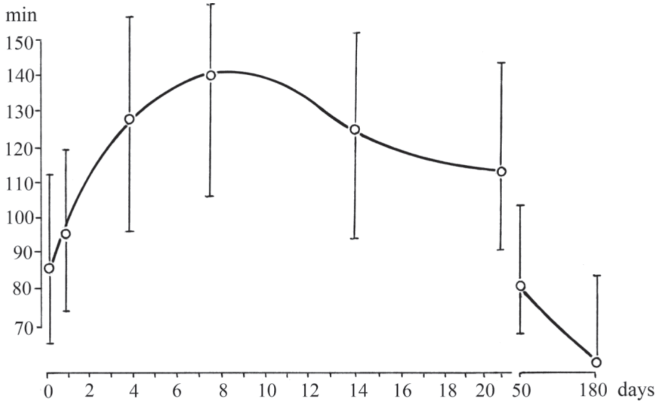

Fig. 10. Age variability of the lifespan of bees at 50 °C and relative humidity of 14 ± 3%.

Seasonal variations in a bee's tolerance to hyperthermia appear to decrease in summer generations of bees as compared with spring generations. As for the bees that emerge at the end of the summer and the beginning of fall, their tolerance to hyperthermia is increased. During overwintering, their tolerance to hyperthermia decreases according to their physiological exhaustion. Therefore bees that overwintered under optimal conditions display a better tolerance to hyperthermia as compared to bees that overwintered under adverse conditions. In particular, bees of colonies that overwintered in a thermostated facility at 0 °C survived at 50 °C for 72 ± 14 min, whereas those that overwintered in the open air at a decreased temperature of –22 °C survived for 1.4-fold less time. Bee diseases have a similar effect in that they also decrease the bees' tolerance to hyperthermia (Eskov, 1995a).

Morphophysiological changes. Depending on the intensity and duration of exposure, hyperthermia induces the development of reversible or irreversible changes in the bee's physiological state. Remote consequences of hyperthermia can modify the age-related dynamics of body weight, water exchange, and so on. Irreversible injuries to internal organs can also take place.

Hyperthermia influences the heart's function and consequently the electrocardiogram (Eskov, 2005). Specific changes in the electrocardiogram caused by bees' heating manifest as the increased amplitude and frequency of electric oscillations. When hyperthermia approaches lethal values in the temperature and duration of exposure, which are different for worker, queen, and drone bees, the generation of electric oscillations is multiply arrested. The duration of cardiac arrest is maximal in worker bees and minimal in drone bees, similar to their tolerance to hyperthermia, and amounts to 16.1 ± 0.73 and 2.9 ± 0.44 s respectively. The restoration of electrical activity after arrest in worker bees continues for 14.8 ± 1.96 s; in queen bees, it continues for 4.1 ± 0.37 s; and in drone bees, it continues for 1.8 ± 0.19 s. In this process, the frequency of electric oscillations initially increases, then decreases again. Before complete cardiac standstill, the frequency of electric oscillations is mostly maintained at a relatively constant level, while the amplitude decreases to a complete fading (Fig. 11).

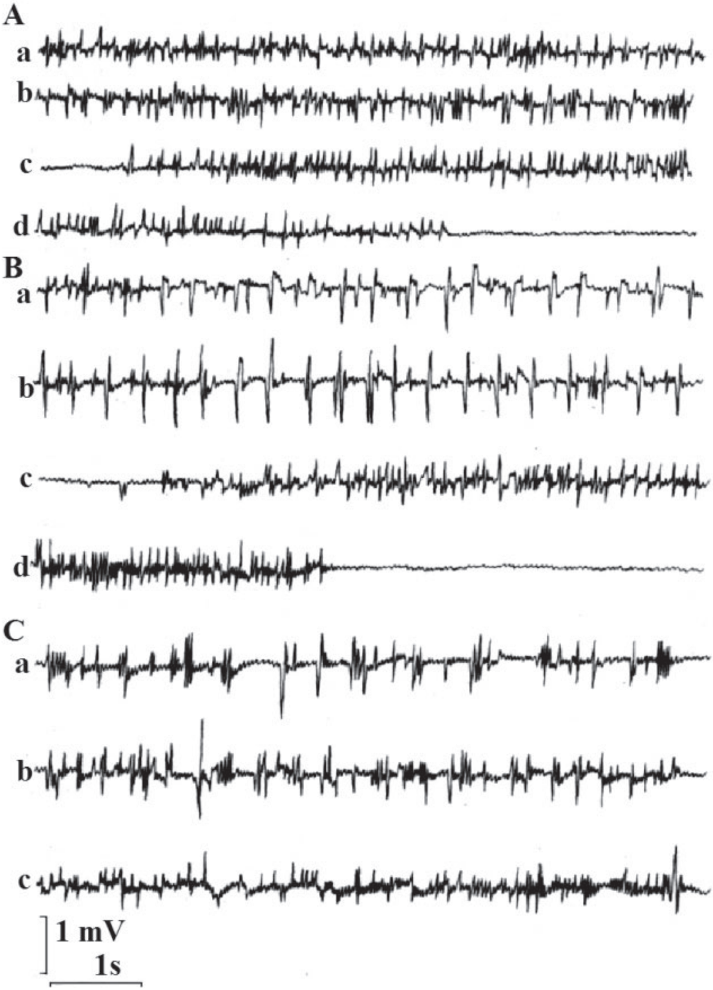

Fig. 11. Temperature dependence of ECG variability: A – worker bees (a – 23 °C, b – 35 °C, c – beginning, d – completion of oscillations at 45 °C (c – beginning, d – continuation of the period of activity after temporary heart activity suspension); B – queens (a – 23 °C; b – 30 °C; c – beginning, d – completion of vibrations at 50 °C); C – drones (a – 4 °C, b – 30 °C, c – 40 °C).

Under the effects of hyperthermia, the energy expenditures for cooling and water evaporation increase. A decrease in the body's water content depends on the bee's age and the extent of the exposure to hyperthermia. The head segment loses water at the highest rate and the abdomen loses water at the lowest rate. At the same exposure to hyperthermia (1 h at 50 °C), the decrease in the water content in the head segments of bees younger than 2 h was 2.7%; in 0.5-day-old bees, the decrease was 6.8%; in 3.5-day-old bees, the decrease was 6.7%; in 7.5-day-old bees, the decrease was 5.3%; and in 14-day-old bees, the decrease was 2.2%. The corresponding values for the thoracic and abdominal segments are 0.4, 1.7, 1.8, 1.9 and 1.1% and 0.6, 1.8, 1.0, 0.8 and 0.3%, respectively.

Irreversible changes in the internal organs increase with the intensification of the heat's impact (Eskov et al., 1986). The thickness of the midget wall, which normally amounts to 117 ± 7 µm, decreases 1.3-fold over 30 min at 50 °C; its folding and secretory activity is also impaired. An increase in temperature increases the injuries over an even shorter time. The wall's thickness decreases 2.5-fold over 10 min at 55 °C; the folding is lost; and the epithelial cells completely lose any signs that demonstrate the accumulation and/or release of secretions. By the moment of death, the wall's thickness is reduced more than threefold (to 28-40 µm), and this is accompanied by the destruction of the bee's muscular layer and the complete destruction of the crypts (Fig. 12).

The effects of high temperatures interfere with the function of rectal glands involved in water transport from the hindgut, which is associated with cooling by evaporation. During hyperthermia, glandular cells and their nuclei swell. Lethal exposures to hyperthermia lead to the destruction of rectal glands and epithelial cells falling out of the hindgut.

Brood. In many species of singly living insects, significant deviations of temperature beyond the limits of its optimal values slow down their development. Within the temperature's optimum limits, there is usually a direct relationship between the rate of development and temperature. Similarly, the embryonic and postembryonic development of a honey bee also depend on temperature, but even more so.

Lethal efficiency. The upper limit of the vital range for the development of worker bees from the prepupae stage is 38 °C (Fig. 13a). At this temperature, an average of 95 ± 3.9% of the developing pupae of working bees are eliminated. The death of most of them occurs in the final phases of the postembryonal developmental stage. About 55% live to middle and old age, and just before leaving the cells, about 40% of the developing bees die. Bees that develop at 39 °C die mostly at the younger pupa stage. No more than 1% of bees live to the stage of pupae of an older age.

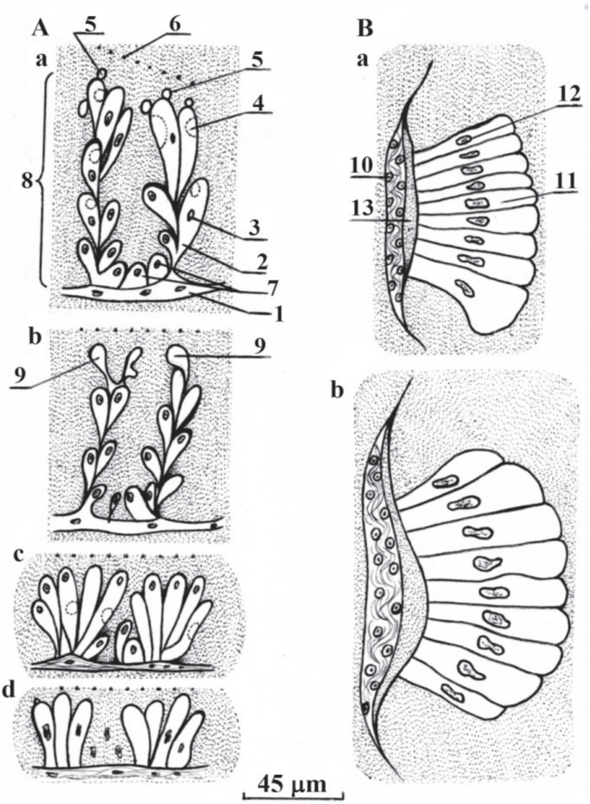

Fig. 12. The effect of hyperthermia on changes in the midgut (A) and rectum (B). A – Medium intestine: a – initial state, b – after 30 minutes at 50 °C, c – after 10 minutes at 55 °C and d – after 25 minutes at 55 °C; B – rectal glands of rectum: a – initial state, b – after 30 min at 50 °C (1 – muscle layer, 2 – intestinal epithelium, 3 – nuclei of epithelial cells, 4 – vacuole, 5 – detached vesicles, 6 – peritrophic membrane, 7 – crypt, 8 – epithelial fold, 9 – dead cells, 10 – outer layer of the rectal gland, 11 – cells of the inner layer of the gland, 12 – nucleus, 13 – gland lumen).

In developing drones, the upper limit of the vital range is at 36.5 ± 0.5 °C. The lethal efficiency of incubation at 36 °C is about 50% and at 37 °C – 100% (Fig. 15b). The death of drones developing at 36 °C occurs mainly at the stage of pupae of the younger and middle ages. At 37 °C, death at the younger age of the pupal stage is 5.0 ± 3.7%, and on average 87.5 ± 9.8% live to an older age.

The drones transfer temperatures exceeding 36.5 °C by fractions of a degree only for a small part of the period of their development. The death of drones developing during the first 6 days at 37 °C and then at 34 °C was 59 ± 13.6%. In the case of the initial 6-day period of development at 34 °C and completion at 37 °C, 78 ± 8.4% of drones survived imago. The reduction of the initial period of development at an optimum temperature by up to two days resulted in all the drones dying, mainly at the stage of middle and older pupae.

The upper limit of the vital range for queens' development is limited to 38 ± 0.5 °C. An increase in temperature from 33 to 38 °C is accompanied by an increase in the rate of death of the developing queens from 6.1 to 35.4%, and at 39 °C all developing queens die (Fig. 13c). Periodic changes (decreases and increases) of temperature within the vital range have different lethal efficacies depending on the limiting values of its changes. With daily fluctuations in temperature over a 12-hour half-period from 31 to 38 °C, the number of deaths of the developing queens will increase by about three times compared to that at 34 °C. Reducing the period of temperature change to 1.5 h is reflected in the reduction of the deaths of queens. But it remains at a level that is approximately double the deaths at the optimum temperature. The decrease in the negative effect of temperature fluctuations in cases of an increase in their frequency is obviously associated with a reduction in the duration of the action of unfavorable extreme values.

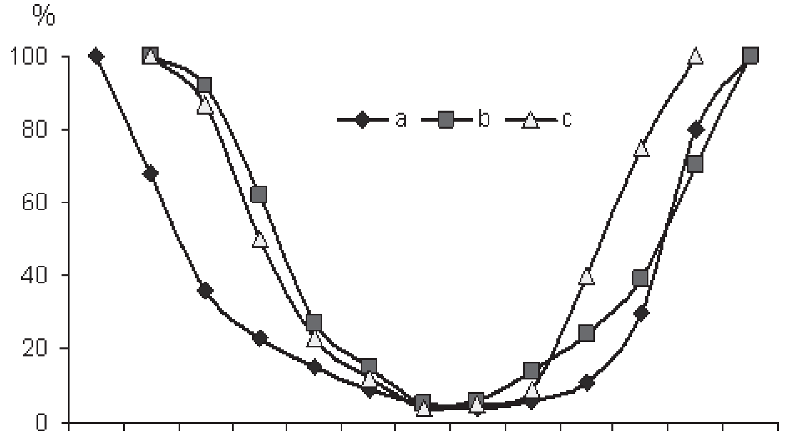

Fig. 13. Temperature dependence of elimination (Y-axis) of worker bees (a), drones (b) and queens (c) developing in sealed cells.

In worker bees, queens and drones, a rise in temperature from the lower boundary of the vital range, at a level of 28–29 °C, to the upper one is reflected in an increase in the length of the proboscis, the diameter of the head, and the size of the mandibles. The dimensions of the wings reach a maximum if they develop in the region of the optimum temperature range. The diameter of the abdominal rings practically does not change, since the width of the tergites decreases with increasing temperature, while the sternites increase. In all bees that develop at the upper limit of the vital range and die at the imago stage before leaving the cells, the wings remain underdeveloped or absent (Eskov, 1995a).

At the egg stage, resistance to hyperthermia does not depend on the gender of the developing embryo. The likelihood of development to the larva stage in fertilized and unfertilized eggs does not differ. For those and others, the death rate at 38 °C is 44 ± 2.7%, and at 39 °C – 100% (Fig. 14).

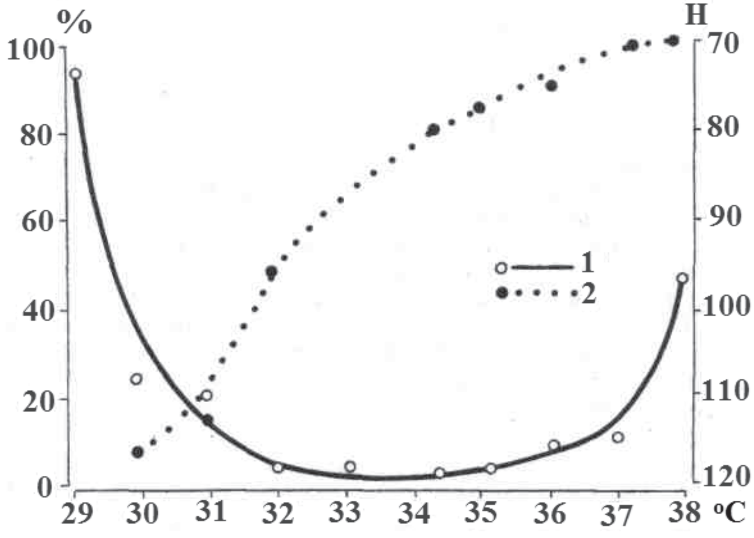

Fig. 14. Temperature dependence of the viability and duration of development of worker bee embryos: 1 – the proportion of hatched larvae (in %); 2 – the duration of embryonic development (in hours).

A short-term but intense warming up of the developing bees gives rise to the occurrence of morphological anomalies. Thus, in 3 ± 1% of the bees which remained at 50 °C for 45 minutes at the stage of the egg or pupa, the proboscis was underdeveloped, which was expressed in a decrease in their length by about a third. Of the structures of the proboscis, the tongue was most changed. Its length decreased by 1.5 ± 0.1 times.

Duration of development. In the optimum temperature region of 34 ± 0.5 °C, the duration of the embryonic stage is 80 ± 4 hours. Raising the temperature to 38 °C leads to a decrease in embryonic development by 1.16 times, and a decrease to 30 °C to an increase by 1.43 times (Fig. 14). Both the fertilized eggs from which the females develop and the unfertilized eggs from which the males develop are subjected to a similar influence by the thermal factor.

Postembryonic development in queens, worker bees and drones in normal conditions and during hyperthermia differ significantly. In queens at the prepupa and pupa stages, the development time at 38 °C is 176 ± 9 hours. This is 19 hours less than under optimum temperature conditions (33 °C).

For worker bees, at 38 °C, the total duration of the prepupae and pupae stages is 235 ± 16 hours. At 34 °C, 55 hours more are required to complete the development to the adult stage. To complete the drones' development from prepupae to adults at a temperature of 36 °C, which has a high lethal efficiency, 322 ± 19 hours is required, which is 26 hours less than at 33 °C.

Consequently, with the temperature approaching sublethal values (here and hereinafter, sublethal values indicate temperature or other factors in which the death rate of individuals is less than 75%), the duration of the development of queens, worker bees and drones is accelerated in the parts of them that have the highest tolerance to hyperthermia. The increase in temperature from optimum to sublethal values accelerates the development of prepupae and pupae of working bees to the greatest extent, and females to the smallest extent.

Hypothermia

Imago stage. The honey bee displays a set of adaptations to cold that allows it to survive for some time under hypothermal conditions. Similar to many insect species that live individually, during cooling, bees enter a chill coma, while their tolerance to freezing is determined by the maximum supercooling temperature, which depends on the state of intra- and intercellular aqueous solutions.

Chill coma. Cooling bees to a certain temperature induces a chill coma, which is characteristic of poikilotherms. In the state of a chill coma, the depth of which depends on the intensity of cooling, locomotion, respiration, and heart function are arrested. This allows the animal to survive a relatively short exposure to cold with minimal energy expenditures (Eskov, 1992; Eskov, Babkina, 1990).

The chill coma threshold in worker bees is at a level of 13.5 ± 0.1 °C. On average, the chill coma at such a temperature in a situation where bees are prevented from leaving the cold impact site begins after 30 min. The decrease in temperature accelerates the beginning of the chill coma. At 0 °C, the bees enter a chill coma after approximately 7 min. Cooling at a higher temperature induces a light coma. For example, bees that fell into a coma at 13.5 °C respond to touch with leg and abdominal movements.

The temperature of an exposure to cold that stimulates a chill coma slightly differs for bees of different intraspecific cohorts. The bee's age and saturation of its lymph with carbohydrates are of importance. In particular, at the beginning of their adult stage (2 ± 1-day-old), at 0 °C, Caucasian honey bees enter a chill coma on average within 3.8 min, while middle European honey bees of the same age enter a chill coma within 3.5 min. At the ages of 17 and 35 days, resistance to freezing by locomotor activation is 1.8 and 1.9-fold longer, respectively.

A decrease in the lymph's saturation with carbohydrates caused by starvation decreases the bee's tolerance to cooling. In particular, bees that carry about 20 mg of carbohydrate feed in their bags at 13.5 ± 0.1 °C enter chill comas after 29.7 min on average and hungry bees in 9.0 min.

The bee's lifespan in a chill coma depends on its physiological state, age, and cooling temperature. At 10 °C, bees retain viability for 65 ± 17 h; at 0 °C, the lifespan of frozen bees reduces 1.2-fold; and at –5 °C, the lifespan decreases 14.4-fold.

Bees in a chill coma cannot activate themselves while they are still under the conditions that caused the coma. Their activation is only possible at a temperature exceeding the threshold value for the chill coma. The activation rate of frozen bees depends on the temperature and duration of cooling. On average, bees exposed to 13.5 °C for 30 min can be activated at 25 °C within 2.8 min. Approximately the same time is required for the activation of bees exposed to 0 °C for 7 min. The time needed for their activation increases on average to 13.7 min after 90 min of cooling at 0 °C. All other conditions being equal, hunger accelerates the activation of bees from a chill coma (Eskov, 1983).

Drastically decreasing metabolic activity depends on the temperature and duration of cooling. The metabolic intensity in chill comas of older bee cohorts changes more considerably than in young bees. At 10 °C, bees of middle and old age consume 67-fold less oxygen than at a temperature of 30-35 °C, whereas young bees (at an age of up to 3 days) consume only

27-fold less oxygen. At 0 °C these differences increase 217-fold and 85-fold respectively.

Hypothermia changes the heart's function, which is evident in the electrocardiogram (Eskov, 2005). The initial phases of a chill coma are preceded by changes in the amplitude and frequency of electric oscillations. The largest changes in their amplitude-temporal structure take place after cardiac arrest; they begin before a complete heart standstill. A gradual increase in the frequency and amplitude of electric oscillations, which reach a certain level in several seconds and then gradually or drastically fade, is characteristic of periods of transient cardiac activation. Several periods of the fading and restoration of the heart's electric activity are followed by the complete standstill of the heart, which coincides with the beginning of a deep coma. Similar changes in the structure of the electrocardiogram take place during the comas of paper wasps (Eskov, 1998) and bumblebees (Eskov, 1999).

The remote consequences of a chill coma manifest as a decrease in the bee's lifespan. The lifespan decreases with an increase in coma exposure and depends on the physiological state and age of the bee. The least sensitivity to the effects of the cold that induces a coma is demonstrated by young bees, and the maximal sensitivity is demonstrated by old bees, especially in individuals after overwintering.

Similarly to worker bees, queen and drone bees that are in a limited space initially respond to cooling with activated locomotion, which manifests as the insect's urge to leave the area with adverse temperature. The presence of several bees with the queen bee has no effect on the rate at which the coma is entered. However, queen bees respond more quickly to exogenous heating than worker bees.

Small groups of bees (up to ten individuals) that entered the coma together with the queen bee do not react to it after the restoration of locomotion. However, a queen bee's return to the nest after cooling to a chill coma attracts bees. The attraction of the queen bee increases with its activation. The largest number of bees is located near the queen bee after the complete restoration of its locomotor activity.

The reproductive function can be impaired in queen bees cooled to a temperature that does not have a pronounced lethal effectiveness (Eskov, 2004a). The probability of this impairment increases with a decrease in temperature and an increase in the duration of exposure. No changes in the reproductive function have been detected in 1–3-day-old queen bees cooled to -0.5 ± 0.5 °C for 1 h. Long cooling at a negative temperature stimulates a delay in ovipositioning. Queen bees cooled for 1 h at -1.5 ± 0.5 °C start to lay eggs after 42 ± 6 h. After cooling at -6 ± 0.5 °C for the same duration, ovipositioning is restored after 65 ± 9 h and continues at a relatively high level for 4-5 weeks. Then, the reproductive activity decreases and the ability to lay fertilized eggs is lost. However, they do not lose viability. The drone bees that develop from eggs laid by cooled queen bees match the average norm in their external traits. The queen bees that lost the ability to lay fertilized eggs after cooling can live for several years (in the maximal observation period, it was 3 years).

Maximum overcooling temperature (MOT). The lowest temperature at which liquid body fractions commence freezing characterizes the animal's tolerance to cold (Kalabukhov, 1946; Ushatinskaya 1957; Lozina-Lozinsky 1990; Eskov, 1884, 1995a). MOT depends on the bee's physiological state and the phase of their individual development. The freezing temperatures for larvae and pupae differ approximately 2.5-fold and, from 0°C to the beginning of crystallization, the overcooling rate varies from 0.16 to 0.04. The rate of cooling to the beginning of crystallization for worker bees displays seasonal and age-related variations (Eskov, 1984). Other conditions being equal, the head and the thoracic and abdominal segments (their contents) have certain differences in their tolerance to freezing. The freezing rate of the head segments in bees of the young age cohort varies from 0.1 to 0.4 °C s^{-1}, while the freezing rate of thoracic segments varies from 0.1 to 0.2 °C s^{-1} and the freezing rate of abdominal segments varies from 0.1 to 0.3 °C s^{-1} (Eskov, Babkina, 1990a, 1990b).

The MOT values display considerable differences for different segments of the bee's body, organs, and tissues. All other conditions being equal, the contents of the head segment have the lowest freezing temperature. During the spring-summer seasons, for bees of a young age, the average freezing temperature of the head segment is -7.7 °C; of the thoracic segment – -5.5 °C; and of the abdominal segment – -6.2 °C. For older ages, the MOT decreases on average by 2.5, 1.8, and 1.6 °C, respectively. The range of MOT variations of drone and queen bees is similar to that of worker bees.

Seasonal MOT variations manifest as a decrease in their value from the spring to early fall. During this period, the average freezing temperature for the head and thoracic segments of the young bee cohort decreases 1.7-fold, and for abdominal segments it decreases 1.6-fold. The MOT values for the head segments of bees of middle and old ages decrease 1.4-fold, the values for thoracic segments decrease 1.3-fold, and the values for abdominal segments decrease 1.1-fold. The highest tolerance to freezing in the spring is demonstrated by the young bees, and the highest tolerance to freezing in the late winter-early spring is demonstrated by old bees.

The MOT varies widely depending on the ecological situation and physiological state of the bee. The tolerance to freezing decreases during starvation. For example, after 6 days of living on internal resources, the MOT increased 1.6-fold for the head segments, 1.3-fold for the thoracic segments, and 1.1-fold for the abdominal segments. However, the presence or absence of feed in the honey bag has no effect on the freezing temperature; the correlation coefficient between the weight of the bag's content and MOT is at the level of 0.01 ± 0.14. On the other hand, freezing depends on feed consumption during the period before cooling, which enables the saturation of the hemolymph with substances (carbohydrate derivatives) that display cryoprotectant properties.

The water content in carbohydrate feed influences the MOT value. That is why the bees that consumed 20% sucrose for 2 weeks had 95.9% of the water content in their rectums, and an average MOT of -2 °C. The bees that consumed 60% sucrose for the same period had 83.5% of the water content in their rectums, and they froze at -4.4 °C. As for the filling of rectums with excrement, their weight has no effect on the MOT of bees in similar physiological states kept under similar conditions.

The fat reserves that are mainly deposited in the bee's fat body are related to their tolerance to freezing. That is why the degradation of a fat body during overwintering influences the increase in MOT, the value of which is maintained at a low level only until mid-wintering, after which it increases. However, a decrease in the freezing temperature from December to late January does not coincide with statistically significant changes in the state of the fat body. This is determined by the strong dependence of the MOT on differently directed random changes in the bee's physiological state, including cooling, hypoxia, and starvation. In particular, the MOT slightly increases in a chill coma but considerably decreases after activation and honey consumption. Hypoxia, which is typical for the natural overwintering of bees, also enhanced a decrease in the MOT.

Correlation of cold adaptations with the dielectric permittivity of the bee's body. The dielectric permittivity of liquid fractions in the bee's body was determined using a measuring system that comprised a remote stroboscopic converter and a pulse generator with a frequency of 108 Hz. The bees cooled from +20 to -25 °C (within the temperature range containing the chill coma and the MOT) were probed with picosecond test signals to record the response with a broadband stroboscopic converter. The time sweep of the signals enabled them to be recorded with an interval of 2.5 ps at a signal duration of up to 10 μs (Eskov, Toboev, 2011a, 2011b).

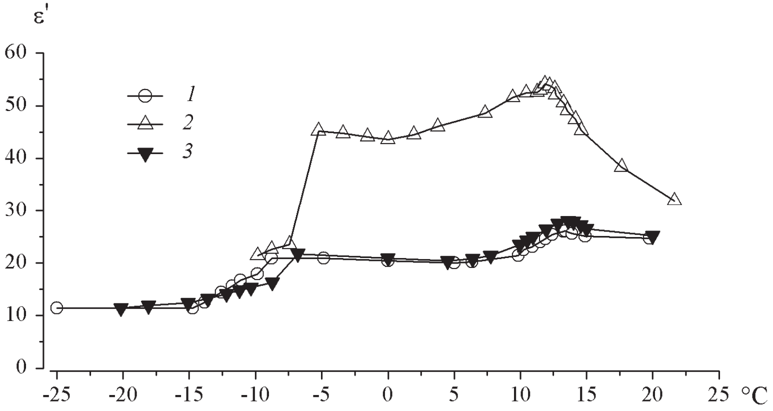

Fig. 15. Temperature dependence of the dielectric permittivity of the bee's body: (1) June; (2) November; and (3) January.

It has been found that the temperature thresholds of chill coma and maximum overcooling, which depend on the seasonal variations in the bee's physiological state, correlate with the relative dielectric permittivity of the bee's body. The bees of spring-summer generations (threshold temperature of chill coma: 12.9 ± 0.4 °C) differed from overwintered bees (13.3 ± 0.3 °C) in the relaxation time of dipole moments. The value of the real part of the dielectric permittivity (ε') for bees of summer generations in a chill coma was 54.2 ± 2.4 versus 25.2 ± 3.7 for overwintered bees.

A decrease in the temperature below the threshold of the chill coma is initially accompanied by a certain decrease in the real part of the dielectric permittivity. The dynamics of this process change when the cooling approaches the temperature when the liquid fractions of the bee's body start to freeze. In the crystallization phase, the ε' value increases in different manners depending on the MOT. The ε' value of summer bees, which on average freeze at -5.1 ± 2.2 °C, reached 44.3 ± 1.6 and, for bees of the fall generations, freezing occurred at -9 to -15 °C, and the value of ε' was almost two-fold lower (Fig. 15).

Presumably, the correlation between drastic changes in the temperature dynamics of dielectric permittivity and threshold values which characterize the tolerance to overcooling is determined by the properties of the water molecules in free and bound (hydration) states. Water molecules display a large dipole moment which is associated with the asymmetric location of two hydrogen atoms relative to the oxygen atom. Water molecules are able to form tetrahedral and more complex network structures of liquid crystal water via hydrogen bonds. An alternating electric field induces dipole polarization due to the presence of molecules with a large dipole moment in water and aqueous solutions.

The orientation of dipole molecules depends on temperature; both the number of oriented dipoles and their relaxation time decrease with an increase in temperature. A delay in the orientation of dipole molecules in the electric field determines the pattern of the dependence of their electric parameters (electric conductivity and dielectric permittivity) on the frequency of the electric field. At the relaxation frequency, both the electric conductivity and dielectric permittivity decrease, while the absorption of the external field energy reaches its maximum value. In the case of dipole polarization, the relaxation time falls into the range of 10–7 to 10–12 s (Kudryashov et al., 2008). The relaxation behavior of the molecules of amino acids, proteins, and water bound to proteins, which manifests as an exponential decrease in the polarization after the switch-off of the external electric field, is observed at frequencies of 108–109 Hz. Note that water, electrolytes, biological macromolecules, tissues, and other structures of biological objects display different relaxation times.

The low variations in the threshold value of chill coma temperatures are likely determined by the relatively large amount of free water, where hydrogen bonds are only formed between its molecules. Presumably, the tight correlation of the freezing temperature values with the biochemical composition of the bound water specified by the seasonal age-related dynamics of the physiological state determines the corresponding variations in dielectric permittivity. Thus, it is possible to assess the change in the tolerance of bees and other animals to overcooling according to the changes in their dielectric permittivity.

Brood. Despite the fact that bee colonies possess effective means of regulating the temperature in the brood zones of the nest (Eskov, 1977, 1983, 1992, 1995a; Heinrich, 1985), in some extreme situations, the brood can be cooled.

Lethal efficacy of hypothermia. A short-term but intensive cooling of the brood has a high elimination efficiency. Of the eggs that were kept at 0 °C for 1.5 ± 0.5 h, 84 ± 4% of bees survived to the stage of imago. Under the influence of the same cooling of the sealed brood of different ages, the death of the pupae was 15.1 ± 3.1%.

The elimination of developing bees occurs in the case of a relatively small decrease in temperature from its optimal value, which is limited to 33–35 °C (Eskov, 1995a). Working bees at the pupal stage survive after a 3-hour cooling to 25 °C. A small elimination efficiency at a level of 0.4 ±

0.2% has an increase in the duration of development at the specified temperature for up to 20 h.

The approximation of the temperature to the optimum values for the brood with an increase in the duration of its exposure does not contribute to the viability of the developing bees. From the brood that develops at 29 °C from the prepupa stage, 38 ± 4.5% of bees do not live to the adult stage, and 62 ± 6.6% die at 28 °C. All developing bees died at 27 °C. In such conditions, about 75% of the developing bees died at the middle-aged pupa stage, and about 20% lived to the oldest. A small part of the bees (no more than 5%) survived to the stage of imago, but none of them came out of the sealed cells.

The reduction in the duration of the hypothermia of the sealed brood is reflected in the reduction in the mortality of developing bees. In the case of development during the first six days at 29 °C and then at 34 °C, the elimination of bees decreased 3.9 times in relation to those developing only at 29 °C. The reduction of the incubation time at 29 °C to 2 days was reflected in an 11.7-fold decrease in the death of developing bees. The higher bee death rate associated with development at 28 °C decreased by 6.2 and 15.5 times respectively by reducing the incubation of brood at this temperature from six to two days.

Bees developing for a short time at 27 °C live up to the imago. If, during the first six days, the brood was at 34 °C after sealing, then at 27 °C the death of the bees was 39 ± 7.8%, and it decreased 3.5 times in the case of an increase in the initial incubation period at the optimum temperature for up to 9 days. Similarly, reducing the initial incubation period at 27 °C helps reduce the death of developing bees. In the case of development after sealing the brood at the specified temperature for 6 days, 66 ± 12.1% of bees died. The reduction of development at 27 °C to 24 hours contributed to an approximately twofold decrease in their death.

Drones' development from a prepupa to an imago at 29.7 ± 0.1 °C, with sublethal values at 29 °C, has an average lethal efficiency. At 28 °C, 96 ± 4% of drones are eliminated at different stages of development, of which 9.4% die at the younger pupa stage and 86.6 ± 7.6% at the middle and older pupa stages.

Like bees, drones shorten the duration of their development at a relatively low temperature, resulting in a decrease in their death rate. If the drones from the prepupae stage developed during the first 6 days at 28 °C, then at 34 °C their death rate was 54 ± 16.5%. Among the drones that developed in the first 6 days at 34 °C and later at 28 °C, the death rate reached 78 ± 17.8%.

Queens differ in their lowest tolerance to cooling during development from the prepupa to the imago (Eskov, Toroptsev, 1978). The death of queens incubated after the queen cells were sealed at 30 °C was about 60%, and at 29 °C it reached 90%. A decrease in the incubation temperature for the indicated minimum value of only 0.2-0.4 °C is reflected in a 100% rate of the death of queens.

Periodic changes (decreases and increases) of temperature within the vital range have different lethal efficacies depending on their limiting values and frequency of changes. Under the influence of daily temperature fluctuations with a 12-hour half-period from 31 to 38 °C, the death of developing queen bees will increase by about three times compared to that at 34 °C. Reducing the period of temperature change to 1.5 h is reflected in the reduction of the queens' death rate. But it remains at a level that is approximately double the death rate at the optimum temperature. The decrease in the negative effect of temperature fluctuations in cases of an increase in their frequency is obviously due to a reduction caused by the thermal inertia of the heating and cooling limiting values of the developing queens.

Duration of development. Hypothermia affects the increase in the duration of embryonic development (Eskov, 1995a). A decrease in temperature from its optimum (33–34 °C) to its sublethal values (29–29.5 °C) is reflected in an increase in this stage from 80 ± 4 h to 118 ± 6 h, or by 1.47 times. The temperature dependence of the duration of the development in haploid and diploid embryos, from which drones or working individuals and queens develop, does not differ.

At the prepupae and pupae stages, at the optimum temperature (34 ± 0.25 °C), the developmental period of working bees is 290 h (max. – 306). At a temperature with an average lethal efficiency (29 ± 0.1 °C), this stage ends on average after 449 h (max. – 479). But with an approach to the sublethal level of hypothermia, the inverse relationship between a decrease in temperature and an increase in the duration of development is violated. In particular, at 28 °C, the development of bees from prepupa to imago is completed on average in 420 hours. This is due to the fact that, as the temperature approaches lethal values, the variability of developmental duration increases and obviously only individuals with high speed survive development.

A change in temperature within the vital range can slow down or speed up development. In the case of brood development during the first 6, 9 or 12 days after its sealing at 27 °C and then at 34 °C, the development time increases, respectively, by an average of 24 ± 3, 48 ± 4 or 75 ± 6 h. Change in exposure sequence-specified temperatures also leads to slower development. If, during the first 9, 6 or 3 days, the brood developed at 34 °C and then until the end of the pupal stage at 27 °C, then its duration increased by an average of 20 ± 2, 51 ± 5 or 99 ± 9 h. In development during the first 6 or 2 days at 34 °C and then at 28 °C, the duration of development increased relative to that at 34 °C by 23 ± 3 or 78 ± 6 h.

The acceleration of development also occurs in the case of initial development at the lower boundary of the vital range and completion at the upper boundary. On average, the duration of development of the brood decreases by 66 hours if it is incubated for the first 6 days at 27 °C and then at 39 °C. However, in such conditions, the completion of the development of bees out of the cells is only 16 ± 6%. Apparently, in such conditions, bees had an advantage in survival, which was distinguished by a high development rate, as a result of which they were less subjected to overheating.

In drones as well as working bees, the variability of the duration of development from the prepupa to the imago increases with the approach to the lower boundary of the vital range. If at 34 °C this stage ends in 341 h (max. – 356), then at 30 °C it ends in 394 h (max. – 412). But at 29 °C, the duration of the development of surviving individuals decreases slightly, averaging 379 h (max. – 397).

Drones developing for some time at a temperature that is below the optimum find that their development slows down. On average, the duration of the development of drones increases by 14 hours, for those which were incubated for two days after sealing the cells at 28 °C and later at 34 °C. An increase in incubation at the indicated temperature of up to 6 days was accompanied by an increase in the developmental duration of 101 ± 9 h. A similar effect on the duration of development is seen in the reverse sequence of changes in the incubation temperature of the brood.

Queens differ from working individuals and drones by their shorter duration of development and, accordingly, by their smaller variability. The decrease in the incubation temperature of queens in sealed queen cells from the optimum value (33.5 °C) to the value that determines the average level of lethal efficiency (30.5 °C) increases the duration of development, on average, from 191 to 243 hours, or by 1.27 times.

Mass of the body and its divisions. The decrease in temperature during the development of worker bees from prepupae to imago from optimal values to 29 °C causes an increase in body weight of 5%. At the same time, the mass of the head sections increases by 2%, and the thoracic – by about 1%. The mass of the abdominal regions increases mainly due to an increase in the filling of the rectum. Their mass exceeds that of the individuals developing at the optimum temperature by an average of 5.6%.

In drones and queens, there is a similar trend in the influence of hypothermia on body weight. As the temperature decreases from the optimal values by 3 °C, body weight increases by 10 and 4.5%, respectively.

The cooling of the brood to 0 °C for 1.5 h was reflected in the decrease in the body weight of the bees that reached the stage of imago. The mass of the head sections decreased by an average of 5%, the thoracic – by 6.2%, and the abdominal – by 8.9%. In the case of a rise in temperature to 25 °C and an increase in the developmental duration at this temperature of up to 20 h, the mass of the head sections decreased by an average of 10.2%, the abdominal – by 7.6%, and the thoracic – by 1.2% (Table 1).

Table 1. The mass of body parts of bees that developed at the pupal stage at the optimum temperature for some time under hypothermia conditions.

Temperature (°C)	Duration of the development	Body part		
		Head	Abdominal	Thoracic
		M ± *m* lim.	*M* ± *m* lim.	*M* ± *m* lim.
34.5	12 days	12.43 ± 0.21 9-14	37.95 ± 0.58 30-44	57.55 ± 1.81 40-72.5
0	1.5 h	11.01 ± 0.23 9-14	35.61 ± 0.84 30-42	52.46 ± 1.54 38-70
25	20 h	11.16 ± 0.41 9-14	37.51 ± 0.91 33.5-47	53.19 ± 2.73 43.5-70

Morphometric variability. The cooling of bees at the embryonic stage of development to 0 °C for 1.5 ± 0.5 h affects the origin of morphological anomalies in 1–1.5% of individuals. This is expressed in the underdevelopment of the mouthpieces and wings. An oral apparatus differs from the norm by a significant shortening of the proboscis. Their length is mainly due to the underdevelopment of reeds, reduced by 36 ± 3%. Anomalies of the wings are expressed in their underdevelopment and the violation of the venation. In this case, it is possible that the veins are underdeveloped and/or new shoots appear in them.

The development during 1.5 hours at 0 °C at the pupal stage was reflected in a decrease in the length of the front wings by an average of 2.5%, and in the hind wings – by 3.3%. Development had a similar effect for 20 hours at 25 °C. The change in the number of hooks (Table 2) largely corresponded to a decrease in the length of the hind wings.

The cooling of the developing pupae has a particularly large effect on the length of the proboscis (Table 2). Under the influence of cooling to 0 °C for 1.5 h, their length decreased by 16.1%. A change in their length that was close to this (at 16.4%) occurred under the influence of development for 20 hours at 25 °C (Table 2).

Table 2. Morphometric signs of bees subjected to cooling at the pupal stage.

Temperature (°C)	Duration of the development	Wing length (mm)		Number of catches	Length of proboscis (mm)
		Front	Back		
		M ± *m* lim.	*M* ± *m* lim.	*M* ± *m* lim.	*M* ± *m* lim.
34.5	12 days	9.34 ± 0.03 9-9.6	6.68 ± 0.03 6.3-7	21.24 ± 0.26 19-24	6.14 ± 0.03 5.5-6.25
0	1.5 h	9.11 ± 0.05 8.5-9.4	6.46 ± 0.22 6.2-7	20.23 ± 0.40 18-23	5.15 ± 0.11 4-6
25	20 h	9.06 ± 0.06 8.3-9.3	6.39 ± 0.05 5.5-6.8	20.47 ± 0.93 17-23	5.75 ± 0.13 4.2-5.9

The development at the pupal stage at a higher temperature approaching the optimal level, as well as under short-term exposure to relatively low temperatures, affected the decrease in the length of the wings and proboscis. In bees developing from the prepupa stage to the imago at 28.5 °C, the shortening of the proboscis is on average 9%, relative to its length in individuals developing in the optimum temperature region. Relatively short queen females are less affected by cooling during development in sealed queen cells. In the queens, under the influence of a decrease in temperature from the optimum value by 3 °C, the length of the proboscis was reduced by about 3%. In drones that developed at the stages of prepupae and pupae at 31 °C, the proboscis' length decreased by 3%, at 30 °C – by 7.4%, and at 29 °C – by only 3.7%. The violation of the tendency to shorten the proboscis with the incubation temperature approaching the sublethal value is obviously associated with the selective elimination of drones, among which individuals with a relatively long proboscis have a survival advantage. This, as noted above, is also characteristic of the temperature dependence of the variability of the length of the proboscis in worker bees and queens.

Thermoregulation

The colonization of a vast area by the honey bee is connected with the acquisition of an intricate set of mechanisms for temperature regulation based on the mobile ethological and physiological complex that makes up changes in the generation, accumulation, and dissipation of heat. However, only aggregating bee swarms and/or their colonies independently of their location are able to utilize these adaptations.

The heating of a bee nest or swarm beyond this nest depends on their physiological state and ecological situation. A bee brood has a strong effect on the activity of thermoregulation. In the presence of a brood, the adult bees try to maximally stabilize the temperature and maintain it, as far as possible, at a level optimal for developing individuals. Along with adult individuals, the bee brood also contributes to the heat balance of the bee nest. The stabilization of thermoregulation within the nest weakens during broodless periods of bee colonies, which are longest in zones of moderate and cold climates (during overwinterings). However, expenditures for thermoregulation increase in accordance with deviations in the external temperature from its optimal value.

The temperature of the bee brood. The zones of honeycombs or their individual cells that house developing individuals are the most thermostable parts of the nest (Hess, 1926; Himmer, 1932; Simpson, 1961; Michener 1974; Bujok et al., 2002; Eskov, 1983, 1992, 1995b, 2003a, 2003b). The composition of a bee colony in its annual life cycle changes considerably. In regions with moderate and cold climates, drone bees account for the major part of the colony in spring and summer. However, neither drone bees nor queen bees are involved in the active thermoregulation of their nest and/or brood; the main contributors are worker bees (Eskov, 1995b).

Depending on the age composition of a bee colony and the number of adult and developing individuals in it, as well as the external temperature, the bee's behavior in the brooding zone can be modified considerably. At a relatively low external temperature (15 °C and lower), the bees are localized to the brooding zone that aggregates around the brood, thereby decreasing the heat loss of the brood and of the overall colony. The bees respond to an increase in temperature above 22-25 °C by activating their locomotion and causing a considerable increase in their density in the brooding zone. The increases in the external temperature and bee flight activity cause a decrease in their number in the nest and on the honeycombs that house the brood.

During the hot season, the thermoregulatory activity of bees is reduced to protect the nest from overheating. Bees provide active cooling for their nest by beating their wings (ventilation). The created airflow intensifies cooling due to water evaporation. Water delivery to the nest increases with an increase in the external temperature above the optimum temperature, the upper boundary of which is 25-28 °C (Eskov, 1995a).

Heat production by the bee brood. The amount of heat generated by the brood depends on the phase of its development, its age, and the temperature (Eskov, 2007). The age-related dynamics of absolute and specific heat generation change with the developmental stage. Low heat generation is characteristic of the prepupal stage. However, upon the pupation of prepupae and during pupal development, heat generation increases, although the body weight decreases. The absolute heat generation by pupae increases from young to old instars approximately twofold, and specific heat generation increases 2.5-fold. Presumably, this is mainly connected with increasing locomotor activation, which reaches its maximum in pupae immediately before the completion of postembryonic development.

The weight of a larva, from the time it hatches from an egg up to pupation, increases approximately 1,500-fold, increasing 50-fold during the first 2 days, but only 1.5-fold during days 5 and 6. The age-related increase in larval body weight is accompanied by an intensified heat generation. However, the specific heat generation (per unit weight) decreases. In particular, 3–4-day-old larvae (with a body weight of 30.3 ± 2.6 mg) at the optimal temperature for their development (34 ± 1 °C) produce on average 0.54 ± 0.07 mW of heat, and 5-day-old individuals with a weight of 122.1 ± 4.9 mg – 0.89 ± 0.08 mW. Accordingly, the specific heat generation of the former is 14.25 ± 0.32 mW/g, and that of the latter is 7.30 ± 0.19 mW/g.

The weight of developing bees reaches its maximal value by the prepupal stage. However, heat generation at this stage decreases considerably. The last instar larvae with a weight of 141 ± 9.3 mg on average generate 0.96 ± 0.09 mW, whereas prepupae with a weight of 143 ± 8.2 mg produce only 0.25 ± 0.04 mW. Thus, the larvae and prepupae that are almost equal in weight under optimal conditions differ approximately fourfold in the amount of generated heat.

Table 3. Heat dependence of larvae and pupae of working bees on air temperature.

Air temperature (°C)	Larvae			Prepupae		
	Average weight (mg) (and its limits)	Heat-segregation (mW)		Average weight (mg) (and its limits)	Heat-segregation (mW)	
		One prepupa	Per 1 g mass		One prepupa	Per 1 g mass
29	115.2 (79-154)	0.48	3.58	150.6 (141-160)	0.18	1.67
34	114.6 (72-152)	0.80	6.94	142.3 (139-150)	0.27	1.90
37.5	115.8 (89-148)	0.88	7.60	148.9 (145-156)	0.31	2.11

At the pupal stage, the age-related decrease in weight is accompanied by an increase in both absolute and specific heat generation. At an optimal temperature, the average heat production of one pupa from the first to the last instar increases 2.1-fold and specific heat production 2.4-fold.

Heat production directly depends on air temperature, independently of the developmental stage (Tables 3, 4). Middle instar larvae respond to a temperature increase of 8.5°C within its vital range (29–37.5 °C) with a 1.8-fold increase in heat production, and prepupae respond with a 1.7-fold increase. By the end of pupal development, the temperature dependence of heat production weakens. The young and middle instars respond to the indicated temperature elevation with 2.0-fold and 2.4-fold increases in heat production, respectively, and the last instar pupae (before the completion of postembryonic development) respond with 1.3-fold increase.

The dependence of heat generation by a bee brood on air temperature is mainly associated with the temperature's effect on the activity of metabolic processes, which change during the period of postembryonic development. An increase or decrease in external temperature by 1 °C in its vital range changes the average specific heat generation of larvae by 0.5 mW/g, while it changes that of prepupae by 0.05 mW/g and that of pupae of different ages by 0.2 mW/g. Therefore, natural cooling, which decreases the temperature of the bee brood, is a major factor in the decrease in larval heat production. Presumably, that is why bees frequently migrate from honeycombs that house larvae and aggregate on the sealed brood (prepupae and pupae) with a decrease in temperature.

Table 4. The dependence of the heat of pupae of working bees on their age and air temperature

Air temperature (ºC)	Age	Average body weight (mg) (and its limits)	Heat-segregation (mW)	
			By one pupa	Per 1 g mass
29	Younger	138.8 (128-144)	0.15	1.15
34	-//-	138.9 (129-143)	0.26	1.86
37.5	-//-	141 (132-149)	0.30	2.13
29	Middle	129.1 (121-144)	0.25	1.97
34	-//-	136.6 (128-144)	0.31	2.30
37,5	-//-	130.3 (121-139)	0.59	4.53
29	Older	122.8 (110-133)	0.48	3.88
34	-//-	120.7 (97-131)	0.55	4.51
37.5	-//-	123.5 (109-134)	0.62	5.17

The high dependence of the heat of developing bees on air temperature is fully consistent with the principle of poikilotherm. This principle is partially modified in adult bees that perform various functions outside the nest and does not correspond at all to the bee colony, especially if there is a brood in its nest. The high stability of thermoregulation in the brood part of the bees' nest allows us to attribute this property of the honey bee to homeothermism.

Thus, developing bees, which require heat, contribute to the heat production of the bee colony. The specific heat production of the brood of different ages is 2–4 mW/g. In a nest containing 10,000 developing bees, their heat production may reach 2–4 W. This can, in part, explain the fact that different temperatures are observed during cooling beyond the brooding zone in the bee nests housing equal bee populations but differing in the number of developing bees. This temperature is higher in nests with relatively more numerous broods (Eskov, 1995a). Presumably, the heat production by the brood is added to the heat generated by the adult bees that heat the brood.

Heat production by adult bees. The heat generation by bees, similar to homeotherms, is connected with locomotor activation or muscle microvibrations somewhat resembling the shivering of mammals. The heating of the bee's body is determined by the amount of heat produced and its dissipation into the environment. In many biological situations, heat generation is a byproduct of locomotor activation. For example, a ventilating bee which cools the nest with the airflow it creates is endogenously heated. The temperature of its thoracic segment at 30 °C can reach 44 °C (Schulz-Langner, 1958). Therefore, independently of the motivation that induces locomotion, the amount of heat generated by a bee is directly correlated with its muscle activity.

Not all the bees that cover the brooding cells are involved in their heating. Some of these bees, which display no pronounced differences in their appearance, remain on the cells with developing individuals and heat them (Eskov, Toboev, 2010). Most frequently, bees are located on the external side of the heated cells, which are sealed with wax caps (Bujok et al., 2002; Stabentheiner, Kovak, 2002; Basile et al., 2008; Eskov, Toboev, 2010a). This is determined by the continuous location of the brood in honeycomb cells during the major part of the reproduction period of worker and drone bees.

The heat production by the bees heating the brood is connected with the microvibrations of the indirect fibrillar muscles. Bees use the same muscles for flight movements of the wings and sound generation (Eskov, 1969, 1979). When functioning in a heat-production mode, the fibrillar muscles do not cause any deformation of the thoracic exoskeleton required for flight and wing movements (Esch, 1960, 1964, 1976; Eskov, Toboev, 2010a).

The heating of the bee's thoracic segment, which warms a brooding cell, alternates with pauses and continues for 0.4 to 5.4 min. From an initial temperature of the cell's surface of 31 to 32.6 °C, it heats at a rate of 0.6 ± 0.3 °C/min^{-1}. The temperature of the dorsal side of the thorax in bees starting to heat cells is 34.2 to 40.3 °C. However, when the thoracic segments are initially well-heated (above 38.5 °C), their temperature decreases during cell heating by 0.4–0.7 °C (Eskov, Toboev, 2010a).

The bee on the surface of a sealed cell does not heat it and the pupa's body uniformly. Usually, before heating begins, the temperature on the inner side of the cell cap is slightly higher than at its bottom. Correspondingly, the temperature of the pupa's head segments exceeds that of the abdomen segments by approximately 0.5 °C. This difference decreases by the end of cell heating and, by the time the bee leaves the heated cell, the temperature at its bottom is higher than the temperature under its cap by 1.1 ± 0.2 °C on average. This is connected with the different heating rates in the segments of a pupa's body. The temperature of pupal head segments increases at a rate of 1.0–1.1 °C/min^{-1}, while the temperature of the thoracic segment increases at a rate of 1.3–1.4 °C/min^{-1} and the temperature of the abdominal segment increases at a rate of 1.5–1.6 °C/min^{-1}; presumably, this is determined by the different degrees of intensification of the metabolic processes in the pupa's body.

Along with the heating of the brood through the external surfaces of honeycombs, bees can use empty cells for this purpose (Eskov, Toboev, 2009a, 2009b; Stabentheiner et al., 2010). In this case, a bee enters the cell by its head, leaving only the very end of its abdomen on the surface. Such bees generate heat for a longer time than those that heat the cells from the surface. The heat generation phases of the bees inside cells reach 24 min, which is approximately four times longer than the maximal thermogenesis periods on the cell surface (Eskov, Toboev, 2010b).

Thermal signaling between the brood and the bees heating it. Thermal (infrared) signaling between developing and adult bees has been demonstrated in special studies using lethal irradiation of the sealed brood (Eskov, 1995a, 1998) and models of worker bee pupae (Eskov, Toboev, 2010b). The irradiation of the brood has made it possible to demonstrate that bees can distinguish between living and dead pupae in sealed cells, removing the latter from the nest by opening the corresponding cells. This is preceded by their intensive heating to 38–39.5 °C. At the same time, bees maintain a normal temperature (at the level of 34.6–35.4 °C) in the adjacent cells with unirradiated pupae.

The above-described data demonstrate that bees that make multiple attempts to heat irradiated pupae cannot establish a normal connection with them. Since the metabolic activity and heat generation by the brood increases, the bees heating it could use at least two signals, i.e. carbon dioxide evolution and/or an increase in heat generation. The bees are able to receive these signals using their specialized carbon dioxide and temperature receptors (Lacher, 1967). A model of pupae whose temperatures change in a specified range has demonstrated that the adult bees that heat the brood use only thermal signals from it.

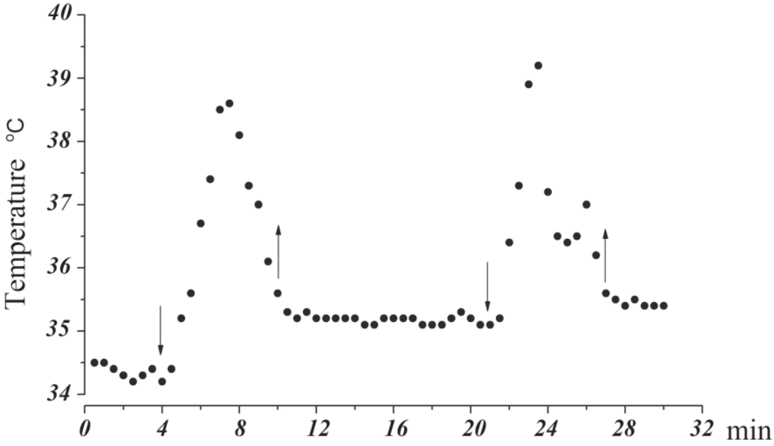

Fig. 16. Radiation temperature on the surfaces of cells with brood in the presence and absence of bees (infrared scanner images): down arrows denote the beginning of heat generation by a bee lying on the cell cap and up arrows denote a bee leaving the heated cell.

The model of the heated pupa is a heating unit fed by a direct current power supply. A linear increase in the powering of the heating unit circuit is provided by a set of resistors. A silicone container (the size of a bee pupa) filled with water was used as a cooled model. Both models were placed into cells with pupae removed beforehand through the bottom, which was then sealed with wax plates. The temperature of the models in the cells and on their surfaces was controlled using temperature microsensors and an infrared scanner (Eskov, Toboev, 2010a).

Bees responded to the heating of the model pupa with an active cooling and wing beats when the cell surface temperature reached 36–37 °C (Fig. 16). The response to the heating of the model diminished when the temperature of the cell surface decreased to 35–35.5 °C. The model that worked in a cooling mode stimulated some bees on the cell surface to heat it. Bees activated by cooling pressed themselves against the cell surface (a typical position during heating) when the cell surface temperature dropped to 32 °C. The temperature of the thoracic segments of bees indifferent to the cooling of the model remained at a level of 30.8–34.8 °C, and that of bees that generate heat remained at a level of 36.6–39.8 °C.

The presence of bees that differ in body temperature in the area of an artificial brooding cell cooling suggests that not all bees are ready to heat the brood, or at least not always. First and foremost, this requires the hereditary motivation that modifies the change in the physiological state of the part of bees to a preparedness to respond to brood cooling and regulate its temperature. The trigger for activating thermogenesis in these bees is the cooling of brooding cells to a certain level. The heat interaction is established in a feedback manner when an adult bee regulates the temperature of a pupa.

Variability of bees' thermoregulatory activity

Depending on the number of adults and developing individuals in a bee colony and on the ecological situation, the temperature in different parts of the bee nest can vary widely. Other conditions being equal, the nest zones occupied by the brood display the maximal independence from the variation in external temperature. The temperature in the nest periphery displays a high variation, especially in the parts with empty honeycombs and stored feed. In the nests of bee colonies in moderate and cold climates, significant changes in the physiological state and behavior of the bees are connected with transitions from brood reproduction to the complete cessation of this process from brood to broodless periods of their annual life cycle (Eskov, 1983, 1992, 1995a; Heinrich, 1985). This is accompanied by the considerable modification of their thermoregulatory activity. In this process, the population of a colony or of a bee group isolated from it is of paramount importance in most diverse biological situations. Small bee groups that aggregate similarly to colonies protect themselves from the adverse effects of the external temperature.

Cold aggregation. Small groups of bees that find themselves outside their home without access to it first react to isolation by some increase in motor activity. Then some of the bees calm down, and other bees begin to gather around them. Their clusters are most often formed on the ceiling of the occupied shelter. Aggregating bees attach to each other with their legs, forming a multi-layered bunch or hemisphere. Regardless of the duration of the isolation, a certain number of bees can move outside the cluster, within it, or on its surface.

The number of aggregating bees and the speed of aggregation depends on their number and the external temperature. In the area of optimum temperature, a group of 10–15 bees does not usually form stable accumulations. The partial aggregation of isolated bees occurs when their number approaches 50 (Free, Spencer-Booth, 1959; Eskov, 1995a). Out of a group of 50 working individuals at 25–27 °C, approximately 25% of the bees aggregate, out of 200 – about 60%, and out of 500 – 90%.

An increase in the number of bees in the group is accompanied by an increase in their thermoregulatory activity. At 24 °C, in the center of a cluster formed by 50 bees, the temperature is maintained at 28.7 ± 0.38 °C, by 100 – 29.5 ± 0.51, and by 500 – 30.9 ± 0.42 °C (Eskov, 1998a). With an increase in the number of bees in the group, the tolerance to cooling increases. In a group of 10 bees at 1–2 °C, their death rate within 20 hours is 63%, of 25 bees – 48%, of 50 bees – 34%, and of 100 bees – 20%.

Relatively high temperatures are maintained in clusters, the number of bees in which reaches 500 ± 50 individuals. At an external temperature of 25–27 °C, the heating of the central zone in such a cluster reaches 31.0–33.5 °C. The bees react to a lowering of the external temperature by increasing the density of aggregation. A decrease in the external temperature at a rate of 1 °C/min leads to a cooling of the central cluster zone by 0.1 °C/min (maximum – 0.3 °C/min).

A group of about 500 bees withstands cooling to 0 ± 0.5 °C for 8–14 hours. Then come chill coma and death. This is preceded by periods of sharp activation of the bees, which begin at the bottom of the cluster. The periods of activation are replaced by calm. Activation cycles repeat every 12–20 min. But often the first phase of activation of cooled bees begins only after 30–40 min.

At the optimum external temperature, which is in the range of 25–27 °C as the bees adapt to life in isolation, their thermoregulatory activity stabilizes. If in the first 7 days the amplitude of temperature fluctuations in the center of the cluster formed by approximately 500 working individuals was 1.6 ± 0.26 °C, then for the next 15–30 days it decreased on average to 0.8 ± 0.14 °C. Regardless of this, sometimes for no apparent reason, the bees have short-term but abrupt periods of activation of locomotion, during which the temperature can rise to 41–43 °C.

The concentration of the carbohydrate feed consumed has a certain effect on the temperature in clusters of bees (Eskov, 1998a). Its increase affects the increase in temperature activity of bees. At 25 °C in the cluster of bees consuming 30% sucrose, the temperature was maintained at 33.3 ± 0.39 °C. The consumption of 60% sucrose was reflected in an average temperature increase of 0.3 °C. At -10 °C, bees consuming 60% sucrose maintained the temperature at 28 ± 1.4 °C for 3 hours. It decreased to 25.9 ± 1.1 °C in the case of the consumption of 20% sucrose.

In the process of adaptation to life in the conditions of light insulation, temperature changes in clusters of aggregated bees acquire a daily cyclical nature. This is expressed in an increase in temperature from night to day.

The temperature in the center of a cluster of bees usually reaches its maximum value by noon and decreases to a minimum with the approach of midnight. The duration of the temperature rise phases is 8–12 hours, and the decrease phases are 11–13 hours long. The periods of thermoregulatory activity represented by large-scale temperature fluctuations are 2.5 ± 0.2 hours.

In terms of isolation from fluctuations in external temperature, air humidity and light, the range of daily changes in the heating of bees depends on their number and external temperature. At 23.0 ± 0.1 °C in clusters containing 200–250 bees, the maximum daytime temperatures are 28.7 ± 0.31 °C on average, and the minimum nighttime temperatures are 26.9 ± 0.29 °C. A twofold increase in the number of bees is accompanied by an increase in daytime temperatures by an average of 3.9 °C, and in nighttime temperatures by 3.6 °C.

Breeding period. *Developing bees*. The temperature in different localization zones of developing bees depends on their localization in the nest and on the honeycomb. An increase in the external temperature from 5 to 35 °C causes an average increase from 34.1 to 36.0 °C at the upper boundary of the brooding zone in a colony comprising 20,000–30,000 bees, an increase from 34.5 to 35.8 °C at the lower boundary, and an increase from 34.9 to 35.6 °C in the center of the nest (Fig. 17).

Changes in daily illumination can cause temperature destabilizations in the brooding zone ofthe nest; this is most likely at an external temperature most favorable for flights. For example, an increase in morning illumination to 20–50 lx at 16–18 °C is accompanied by an increase in the inner nest temperature in the brood located at the side of the entrance by 0.7–0.8 °C. In cool weather that prevents bees from flying out of their hives, no increase in temperature is observed in the brooding zone.

Under conditions favorable for bees to fly out, the productivity of a feeding site has a certain effect on the daily temperature variation. The inner nest temperature can be elevated by 1.8–2.3 °C, but at the expense of the activation of the bees involved in feed harvesting. The temperature is established at levels that mainly depend on the external temperature after favorable conditions for feed harvesting have ended.

The daily heating dynamics of the nest zones that house the brood directly depends on the variations in the external temperature in the range of 34–35 °C. At a high external temperature that reaches or exceeds 37 °C, the direct dependence of the inner nest temperature in brooding zones on the external temperature is destroyed. For example, the temperature in the nest center occupied by the brood elevates from 35.7 to 36.4 °C during periods of hot weather over several days at the beginning of the day with an increase in the external temperature from 22 to 32 °C. Further growth in the external temperature to 36 °C up to midday is accompanied by an average decrease in the inner nest temperature by 0.15 °C. When the external air temperature approaches 40 °C (after midday), the temperature variations in the center of the nest increase and their mean value decreases to 35.3 °C (Eskov, 1995a).

With the changing stages of the development of the brood, the attitude of the adult bees to it changes. The larvae need not only a certain temperature for development but also constant trophic support. After the larvae turn into prepupae and pupae, they develop in sealed cells. At these stages, they need a certain temperature and gas environment enriched with oxygen.

The transformation of larvae into prepupae and pupae, as a rule, is associated with some decrease in temperature at the surface of the cells (Eskov, 1993). The amount by which the temperature changes after sealing the brood depends on its localization in the nest, the number of worker bees in the colony, and the external temperature.

For example, in nests of colonies containing 26 ± 4 thousand adults and approximately the same number of developing bees, the larvae located on the extreme cell on the side facing the side wall of the hive or the honeycomb with fodder stocks at 1–29 °C have an average temperature of 35.1 °C. After the larvae were transformed into prepupae and pupae, the temperature decreased by an average of 1.1 °C. The larvae in the center of the nest developed at a temperature of 35.3 °C, and after their transformation into prepupae and pupae it decreased by 0.2 °C ($P \geq 0.95$).

An increase in the number of bees in colonies resulted in a decrease in temperature change after sealing the brood. In colonies in which the number of adult bees was 40 ± 5 thousand, the temperature change after sealing the brood on the periphery of the nest was equal to an average of 0.8 °C, and in the center it did not exceed 0.1 °C.

With a decrease in the number of bees in the colony, the magnitude by which the temperature drops after sealing the brood increases. For example, at 6–18 °C, larvae located in the central part of the nest of the colony containing 2.3 ± 0.11 thousand bees had a temperature of 34.1 °C, and in the colony with 19 ± 1 thousand adult individuals – 35 °C. After sealing the brood, the temperature decreased by 1.0 and 0.2 °C, respectively.

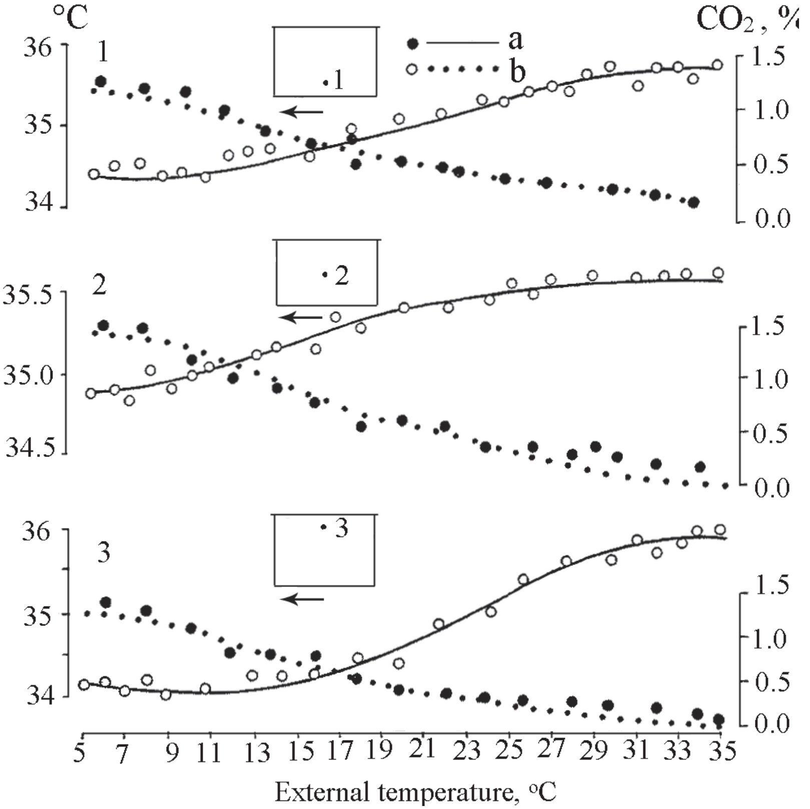

Fig. 17. Dependence on the external temperature (X-axis) of the heating of the brood (a) and carbon dioxide content (b) in different zones of the central part of the nest (1–3) occupied by the brood.

Coolings increase the effect of the number of bees on the temperature of open and sealed broods. In particular, under the influence of a change in the external temperature from 16.5 ± 1.5 to 8 ± 2 °C, the temperature of the larvae in the nest of a colony containing 2.3 ± 0.11 thousand bees decreased by 0.5 °C, and the temperature of the pupae – by 0.9 °C. In the nest of a colony containing 19 ± 1 thousand bees, the temperature of the larvae and pupae decreased, respectively, from 35.2 to 34 °C and from 35 to 34.7 °C, or by 1.2 °C and 0.3 °C.

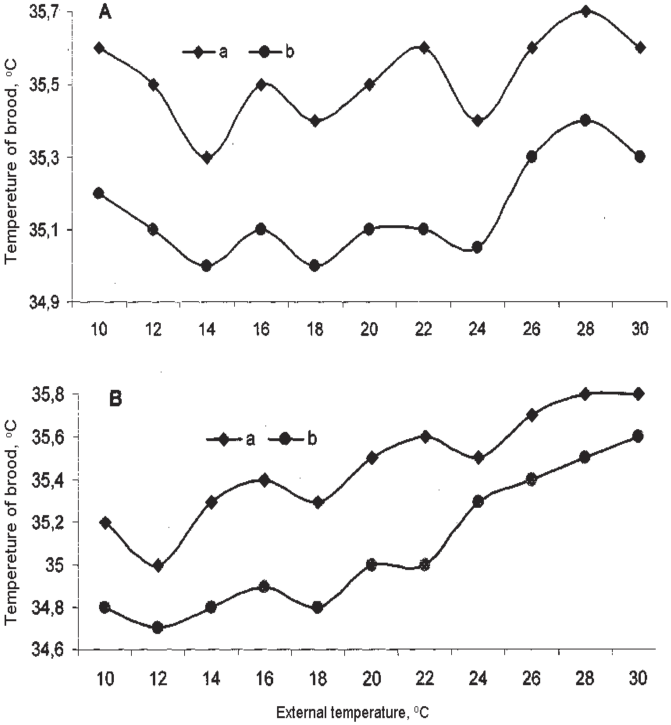

Fig. 18. The temperature of the open (a) and sealed (b) broods, localized in the center (A) and on the periphery of the bees' nest (B).

The effect of sealing the brood on lowering the temperature depends on its localization in the nest (Fig. 18). In particular, in the nest of a colony containing about 27 thousand bees, when the external temperature fluctuated from 12 to 28 °C, the larvae that were in the lower part of the honeycomb had a temperature of 35.0 °C, in the central part – 35.2 °C, and at the top – 34.9 °C. After the brood had been sealed, the temperature in the central and upper parts of the honeycomb dropped on average by 0.3 °C, and in the lower part – by 0.65 °C.

The temperatures of the larvae and pupae, simultaneously developing in similar zones of the nest, differ when it overheats. For example, at 36 ± 2 °C in a colony containing about 28 thousand bees, the average sealed brood located in the middle part of the honeycomb had an average temperature of 36.5 °C (maximum – 36.8 °C), and larvae – 36.8 °C (maximum – 37.5 °C).

Developing drones. On honeycombs with developing drones, there are some differences in temperature from those with developing bees. At 6–25 °C in a nest of a colony containing about 30 thousand bees, the temperature of the drone brood localized in the central part of the honeycombs was 35.2 ± 0.14 °C. At 13–31 °C, the temperature of the developing drones in the central part of the honeycombs was 35.4 ± 0.16 °C, dropping on the periphery by an average of 0.3–0.4 °C.

Spring-summer coolings, suspending the departures of bees from the hive, do not have a significant effect on lowering the temperature in the zones of the nest occupied by developing drones or bees. When the external temperature falls from 25 to 6–7 °C in a colony containing about 27 thousand bees, the temperature at the drone brood in the nest's center decreased by an average of 0.26 °C, above the center – by 0.51 °C, and in the lower part of the cells – by 0.79 °C. A similar drop in temperature occurred in the bee brood.

In the range of external temperature fluctuations from 8 to 36 °C, the temperature in the arrival zone of the localization of developing drones increases by an average of 2.8 °C, in the central part – by 2.0 °C, and in the upper part of the cells – by 2.9 °C. The differences in temperature in these zones increase with the cooling and overheating of the nest, and at 25–30 °C it is set at the same or similar levels (Fig. 19).

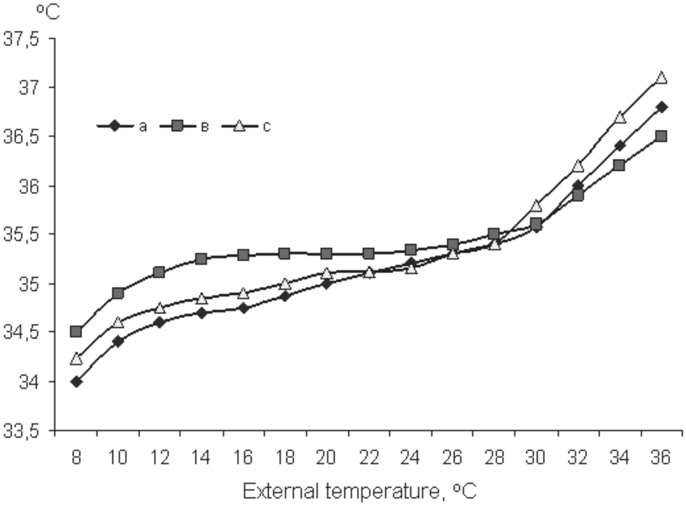

Fig. 19. The influence of the external temperature on the temperature of the drones developing in different zones of the nest: a – from the entrance notch, b – in the center, c – above the center in the upper part of the honeycomb (external temperature on the x-axis).

During the reproduction season of drones, the number of bees in a colony may vary significantly for various reasons. The temperature in the brood zones of the nest depends on this. When the external temperature fluctuated from 10 to 30 °C, there were no significant differences in the average temperature of the developing drones in the nest of a colony containing 32 ± 3 or 16 ± 2 thousand bees, equaling, respectively, 34.89 and 34.85 °C. But the increase in external temperature by 20 °C had a different effect on the temperature in the brood zones of these colonies. In a colony with a relatively high number of bees, the temperature increased by an average of 0.5 °C, and with half as many bees – by 1.1 °C ($P \geq 0.999$).

In colonies with very low numbers of bees, the temperature of the drone brood is strongly dependent on the external temperature. In particular, the temperature in the nests of colonies containing about 2 thousand and 20 thousand bees differed significantly. Under the influence of lowering the external temperature from 20 to 10 °C, in colonies containing 2 thousand bees, the temperature of the developing drones decreased by 1 °C (from 31.3 ± 0.12 to 30.3 ± 0.11 °C), and for bees – by 1.5 °C (from 32.7 ± 0.14 to 31.2 ± 0.12 °C). At the same time, in colonies containing about 20 thousand bees, the temperature decrease in drones averaged 0.1 °C (from 33.9 ± 0.13 to 33.8 ± 0.12 °C), and in bees – 0.6 °C (from 34.5 ± 0.13 to 33.9 ± 0.12 °C).

Developing queens. The reproduction of queens, unlike bees and drones, does not have a strict seasonal cyclicity. For a certain season, there is a reproduction of queens only during the period of sociotomy (breeding of bee colonies). But not all colonies breed every year. In addition, in some races, the breeding instinct of colonies is very weakly expressed (for example, in gray Caucasian mountain bees). Regardless of this, the most likely cause stimulating the reproduction of queens is related to their death or non-compliance with the physiological needs of the colony. This is usually caused by a lack of secretion of the queens' substance.

The localization of the developing queen bees in the nest depends largely on the reason that prompts their reproduction. During the preparation of colonies for breeding, the bees rebuild the queen cells mainly on the periphery of the honeycomb. They have similar placement most often when replacing the old queen with the colony. In the case of its sudden death, the bees do not have the opportunity to rebuild the queen cells in advance for the queen to lay eggs in them. In such situations, the choice of a place for the development of queen bees is limited to the localization of the larvae of worker bees.

The temperature of the queen cells depends on their localization in the nest, the external temperature and the number of bees in the colony. Relatively high temperatures are maintained as a rule in the queen cells located in the center of the nest. In this zone at 6–27 °C, the temperature at the queen cells is maintained at 34 ± 0.12 °C. On the lower side of the honeycomb, the temperature at the queen cells decreases on average by 0.7 °C, and on the upper side – by 1.3 °C. Following an increase in temperature from 6–9 °C to 24–27 °C, the temperature at the queen cells located in the lower part of the honeycomb increased from 33 ± 0.11 to 34.1 ± 0.12 °C, in the center from 33.1 ± 0.1 to 34.7 ± 0.12 °C, and at the top from 32.2 ± 0.09 to 34.1 ± 0.13 °C.

The temperature of queen cells depends on the number of bees in the colony. The lowest dependence on the number of bees in the colony is demonstrated by the temperature of the queen cells localized in the central and lower parts of the nest. For example, with daily fluctuations in the external temperature in the nests of colonies containing approximately 35 and 14 thousand bees, the temperature of the queen cells located in the central and lower (from the entrance side) parts of the cells did not differ much and averaged 34.3 °C. In the queen cells located in the upper part of the nest on the side facing the back wall of the hive, the temperature in a colony with a larger number of bees exceeded the value of a colony with a smaller number by 0.4 °C on average.

In colonies with a very low number of bees, fluctuations in the external temperature have a strong effect on lowering the temperature of queen cells after they are sealed. As the temperature increased from 6 to 18 °C in nests of colonies containing about 2.3 thousand bees, the temperature of the unsealed queen cells increased from 33.4 ± 0.46 to 34.1 ± 0.56 °C, and after sealing from 32.4 ± 0.39 to 33.6 ± 0.53 °C. For the colony containing about 19 thousand bees, the temperature of the unsealed queen cells changed from 34.7 ± 0.58 to 35.4 ± 0.54 °C, and that of the sealed ones from 34.6 ± 0.39 °C to 35.3 ± 0.54 °C.

Adaptations to wintering. Cooling during the fall-winter period stimulates bee aggregation near the zone of maximal heating (heat center). The alternation of the phases of relative rest and active heat generation destabilizes the temperature in various zones of bee aggregation considerably. Their unordered migrations within a swarm cause local air mixing, which influences the dynamics of air flows (Eskov, 1995a).

The external temperature has the most pronounced effect on the warming of the heat center at the beginning of overwintering, changing its temperature in a rather wider range (Fig. 20). In particular, the temperature variations in the heat center of a colony comprising about 20,000 bees reached 2.5–2.7 °C in November–December and 1–1.6 °C in February–March. Correspondingly, the correlation coefficient between the external temperature, varying in the range of -17 to +11 °C, and the warming of the heat center decreased from -0.81 ± 0.10 to 0.13 ± 0.04. During the entire overwintering period, the mean correlation coefficient between the temperature in the heat center and the periphery of the bee swarm located above it was 0.82 ± 0.11 (Eskov, Toboev, 2009, 2010).

The correlation between the variations in the external and maximal inner nest temperatures depends on the number of bees in the colony. In relatively small colonies (approximately 10,000 individuals), a strong negative correlation between the analyzed temperatures is observed only at the beginning of the overwintering period (at the initial stages of adaptation to the fall cooling). Starting from December, the correlation coefficient decreases to -0.36 ± 0.12. In the colonies that comprise approximately double the bee population (≈18,000 individuals), the temperature variations in November–December reached 0.8–2.7 °C, and the correlation coefficient was -0.84 ± 0.09. In the second half of the overwintering period (from January), the colonies responded weakly in their changes in thermoregulatory activity even to considerable changes of the external temperature.

Independently of their population, bees respond to cooling with a decrease in the swarm surface by compaction, which reaches its maximum at the interface with the cold air. Note that heat flows in various directions differ due to the inhomogeneities formed in the swarm structure. The total heat flow from its surface depends on the effective external surface, i.e. on the area through which the heat is given up. It is always smaller than the geometric surface, and its value significantly depends on the external temperature, the localization of bees to various spaces between honeycombs, and the distance to the entrance opening. The heat between the inner hive space and the environment is mainly exchanged through this opening.

In the optimal temperature range of -3.0 to +8.8 °C, the intensity of the heat flow per 1 cm^2 of the surface is weakly dependent on the number of bees in a swarm. In the colonies comprising 8,200–19,500 bees, the heat flow intensity is 2.05–3.21 × 10–3 W/cm^2; note that the flow size depends on its direction. In the aforementioned range, it is larger towards the upper surface and smaller towards the bottom. In the optimal zone, no considerable change in the temperature of the heat center is observed, which is determined by the similarity in the heat load (heat generation) per bee (the mean value falls in the range of $6–8 \cdot 10^{-4}$ W).

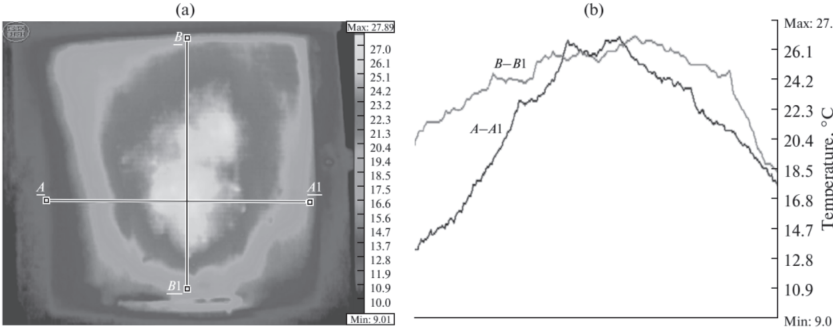

Fig. 20. Distribution of heat fields in the central bee swarm between honeycombs at -2 °C: (a) thermogram and (b) temperature profiles for sections A–A1 and B–B1.

The deviation of the external temperature from its optimum for the colony is usually accompanied by the increased warming of the heat center (Fig. 20a). Here, the number of aggregated bees is important; a decrease in their number destabilizes the heat content and elevates the heat losses, which are compensated for by additional feed consumption. In particular, the average main exchange in the optimum zone for a colony of approximately 8,000 bees is 3.28 W (47 cal/min). The cooling to -15 °C for 120 min causes a 2.6-fold increase in the heat production of the overall colony, with a heat load for each bee of 6×10^{-4} to 1.1×10^{-3} W.

The external temperature and number of bees in the nest influence their distribution in the adjacent spaces between honeycombs at the beginning of the overwintering period. The highest aggregation density is attained in the lower part of each bee swarm between honeycombs, which is determined by the effects of cooling. The aggregation density decreases closer to the heat center (the zone of maximal heating). Relatively low bee compaction is observed above the heat center, which is determined by their heating by ascending heat flows (Fig. 20b).

The temperature gradients directed from the heat center towards the upper and lower boundaries of bee swarms differ considerably in both their absolute values and their variations. The temperature gradients change insignificantly, not exceeding 12 °C, during the overwintering in the upward direction, whereas in the downward direction they constantly vary and reach 12–28 °C. At -20 to -18 °C, the difference between the external and bee body temperatures is maintained at the same level in the lower part of the swarm.

The heating of different body parts of the bees in the swarm periphery depends on their localization and the external temperature. When this temperature varies from -1 to -13 °C, the thoracic segment usually displays maximal heating. The average temperature of this segment differs by several tenths of a degree from the temperature of the head and abdominal segments. However, the temperature of the head segment sometimes approaches or slightly exceeds the thoracic temperature. As for the abdominal temperature, it is always lower than that of the thoracic segment (Eskov, Toboev, 2009a).

The decrease in the external temperature stimulates an increase of the body temperature of the bees in the periphery of the swarm. The most pronounced heating is observed in the lower part of the swarm, which is usually subject to the most intensive cooling. In particular, when the external temperature decreases from -1 to -21 °C, the average thoracic temperature increases by 3.0 °C, the head temperature decreases by 2.6 °C, and the abdominal temperature decreases by 2.4 °C; the corresponding values for the upper part of the nest are 1.5, 0.6, and 0.9 °C. The maximum temperature of the bees in the bottom part of the nest at -13 °C was 27.9 °C and the minimum was 7.5 °C.

With a decrease in the external temperature, the proportion of bees whose thoracic segments are better heated than the head increases. At -1 °C, the difference between the temperatures of the head and thoracic segments reaches 5.7 °C; at -13 °C, it reaches 8.5 °C; and at -21 °C, it reaches 10.3 °C; the proportions of bees with a thoracic temperature that exceeds that of the head by at least 2 °C are 25.4, 36.0, and 47.2%, respectively. However, a decrease in the external temperature is accompanied by a decrease in the bees cooled to the temperature that induces a chill coma on the lower surfaces of bee swarms. In particular, their mean fraction at -1 °C is 52.4 ± 4.9%; at -13 °C, it is 45.7 ± 4.1%; and at -21 °C, it is 32.1 ± 3.3%.

The body temperature of the bees localized at the zone of the heat center varies in a narrower range. In particular, the temperature of the head segments at -20 °C varied in individual bees from 26.8 to 34.3 °C; the temperature of the thoracic segments varied from 24.2 to 36.0 °C; and the temperature of the abdominal segments varied from 24.6 to 33.4 °C, with averages of 29.8, 30.7, and 29.3 °C respectively.

Thus, the stability of thermoregulation during the broodless period in the life of a bee colony decreases compared with the period when their nest houses developing bees. In the nest of overwintering bees, the individuals localized in the lower part of the swarm experience the most pronounced cooling. By preventing the cold air from entering the swarm, they protect the overall colony from overcooling.

Exogenously and endogenously dependent temperature fluctuations. Independently of the changes in external temperature, the inner nest air temperature in the spaces between honeycombs fluctuates and displays short-term drops. It sometimes reaches 5.5–7 °C. Most frequently, these fluctuations of the air temperature inside the nest, following at 7–16-min intervals independently of the time of the day, do not exceed 0.5–1.3 °C.

The time structure of large-scale fluctuations in the thermoregulatory activity changes both diurnally and in the annual cycle of the bee colony's life; differences are distinctly seen in the spring-summer and fall-winter seasons, which are most likely associated with seasonal variations of the bees' locomotor activity. In regions of moderate and cold climates, this activity reaches its maximum in the late spring, is retained at a high level until the fall, and is close to its minimum during overwintering. However, the average duration of large-scale temperature fluctuation periods increases from the spring-summer to the fall-winter phase of the bees' annual life cycle from 2.15 ± 0.10 to 2.4 ± 0.3 h. Their daily duration changes in approximately the same range. In the daytime of the spring-summer season, it amounts to 2.3 ± 0.1 h and, at night, it is 2.1 ± 0.1 h; the corresponding values for the fall-winter season are 2.5 ± 0.5 and 2.2 ± 0.3 h.

The periodicity of the large-scale variations in the bees' temperature activity displays no pronounced correlation with the changes to the external temperature. The stabilization of the external temperature also has no effect on the established rhythm of the inner nest temperature fluctuations, as has been demonstrated for bee colonies that initially overwintered in the open air in the temperature range of +2 °C to -19 °C and were then transferred inside to a temperature of +4 to 4.4 °C. The adaptation of bees to these conditions was first accompanied by the phase shift of the thermoregulatory activity peak, the fluctuations of which returned to their initial values in the open air after 3 days (Eskov, Toboev, 2009a).

The colonies that differ in the number of their worker bees may differ in the rhythmic patterns of their large-scale temperature fluctuations. However, in the presence of a heat contact (the effect of the heat produced by one colony on another), the colony with a smaller bee population synchronizes its large-scale fluctuation rhythm with the larger colony.

The fluctuations in thermoregulatory activity are also characteristic of isolated swarms of aggregating bees. Their aggregation activity depends on the population and external temperature (Free, 1958; Michener, 1974;

Eskov, 1983). The partial aggregation of isolated bees commences when their number approaches 50 individuals. A further increase in the number of bees increases the aggregation activity. About 90% of approximately 500 isolated bees aggregate at a temperature of 24–27 °C and 100% aggregate at 10 °C. The increase in the population of the isolated bee group is accompanied by an increase in the temperature inside their swarms. At 24 °C, the temperature in the center of a swarm formed by 50 bees is maintained at a level of 28.7 ± 0.38 °C, while the temperature in a swarm of 100 bees is 29.5 ± 0.51 °C, and the temperature of a swarm of 500 bees is 30.9 ± 0.42 °C.

The quality of food has some influence on the temperature in bee clusters (Eskov, 1995a). The concentration matters in terms of the consumption of carbohydrates dissolved in water. The concentration increase is reflected in the rise in the temperature that is maintained by aggregating bees. In particular, at 25 °C, in a cluster of about 500 bees consuming 30% sucrose, the temperature is maintained at 33.3 ± 0.39 °C. It increases by an average of 0.3 °C when a 30% sucrose solution is replaced with a 60% solution (Eskov, 1995a, 2016).

Similarly to the temperatures inside bee nests, the temperatures in bee swarms are also subject to fluctuations (Eskov, 1995a); their amplitude decreases during adaptations to the new isolation conditions. In particular, the amplitude of temperature variations in the center of a swarm comprising approximately 500 bees during the first 7 days was 1.6 ± 0.26 °C and decreased on average to 0.8 ± 0.14 °C in the following 15–30 days. Sometimes, considerable short-term bursts of locomotor activity and increases in temperature reaching 41–43 °C took place without any evident reason.

During the adaptation to isolation from light, the changes of temperature in aggregated bee swarms acquire a circadian pattern (Eskov, 1998b). It appears as an increase in temperature from night to day (Fig. 21). Usually, the temperature in the center of a bee swarm reaches its maximum by noon and decreases to its minimum near midnight. The duration of phases of temperature increases is 8–12 h, and the duration of the temperature decrease is 11–13 h. The periods of thermoregulatory activity with large-scale temperature fluctuations are 2.5 ± 0.2 h.

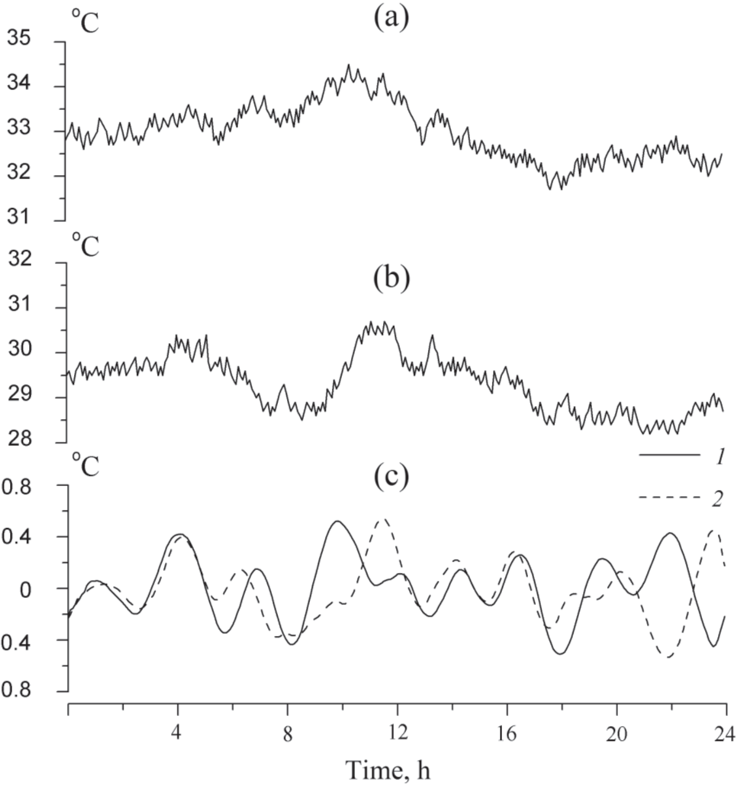

Fig. 21. Daily dynamics (abscissa axis) of the heating of the centers of clusters of bees containing about 500 working individuals, and the normalized values of large-scale temperature fluctuations at an external temperature of 4.2 ± 0.5 °C (a – bees from the colony wintered under the open sky; b – indoors with an illumination of about 0.01 lux and a temperature that varied from 1 to 3 °C; c: 1 – bees from colony "a", 2 – from colony "b").

In conditions of isolation from fluctuations in external temperature, air humidity, and light, the range of daily changes in the heating of bees depends on their number and external temperature. At 23.0 ± 0.1 °C in clusters containing 200–250 bees, the maximum daytime temperature averaged 28.7 ± 0.310 °C, and the minimum nighttime temperature – 26.9 ± 0.29 °C. A twofold increase in the number of bees is accompanied by an average increase in daytime temperature of 3.9 °C, and an increin nighttime temperature of 3.6 °C.

Mathematical Modeling of the Temperature of Field Distribution in Insect Winter Clusters

Bee colonies in moderate and cold climates can winter at ambient temperatures from -35 to +45 °C, which does not correspond to the individual cold resistance of bees. Their individual cold tolerance is determined by the freezing temperature of body liquids of -7 to -16 °C (Eskov, 1995a). The ability of the species to survive the long winter is based on a complex of hereditary programmed eco-physiological reactions stimulated by the cold. Protection against life-endangering cooling is attained through the formation of ordered clusters that ensure the accumulation and rational use of heat (Eskov, 1984, 2003, 2016).

The construction and study of mathematical models of temperature field distribution are based on investigating the natural processes of heat production and loss taking place in bee clusters (Eskov, Toboev, 2009b). The bees were kept in special hives with hinged combs that could be opened. The temperature field distribution and heat production in the clusters were examined with the infrared imagers IRTIS-2000 and ThermaCam S3000.

According to the results of the thermogram analysis, an alternation of periods of relative rest and active heat generation was revealed. This was accompanied by a disordered migration of bees and a destabilization of thermal processes (Fig. 22). But with a decrease in the external temperature, the number of active bees at the lower surface of their clusters increased.

In the heat core of the model, the temperature range 24–32 °C is regarded as the preferred one whereat the bees can feed and migrate freely. In the bees forming the bottom of the cluster periphery (the most cooled part), the body temperature dropped to 8–13.5 °C, which initiates the cold torpor and minimization of metabolic processes.

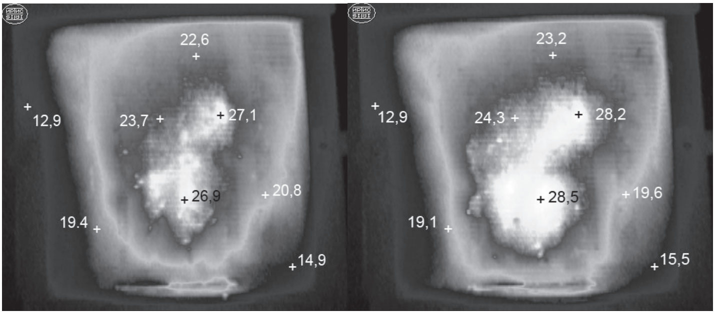

Fig. 22. Images of the thermal fields in a bee winter cluster taken 60 s apart.

The premises for modeling the thermoregulation mechanisms were as follows. The bees' winter cluster is regarded as a spherically symmetrical structure within which heat transfer takes place by through-body conduction and partly by convection through air gaps between bodies. The main stimulus to which the clustering bees react by changing the volume they occupy is the ambient temperature. The latter and the localization in the cluster determine the behavior of every bee and its part in thermoregulation. The threshold for the stimulation of heat production by 'shivering' (microvibrations of flight muscles) is about 18 °C, whereas 13.5 °C corresponds to the onset of superficial cold torpor, which drastically reduces the metabolism. In such a state, the bee can survive only for a few days (Eskov, 1984).

Assuming the spherical symmetry of the model cluster, the temperature field is unidimensional, i.e. heat spreads only along the sphere's radius. The temperature T(r, t) and density p(r, t) of the bees are functions of distance r from the center of the model to the point considered within the cluster and of time t. With these and the above modeling conditions, the classical thermal conductance equation takes the form

$$c\frac{\partial T}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\lambda(\rho)\frac{\partial T}{\partial r}\right) + \rho f(T) \qquad (1)$$

with boundary conditions

$$\begin{cases} \left.\dfrac{\partial T}{\partial r}\right|_{r=0} = 0; \\ \left.\left(\lambda(\rho)\dfrac{\partial T}{\partial r}\right)\right|_{r=R(t)} = \alpha\left(T_{\text{вн}} - T\left(R(t)\right)\right), \end{cases} \qquad (2)$$

where c is the specific heat capacity, (p) is the conductivity coefficient, f(T) is a function of the individual bee's metabolism, a is the heat emission coefficient depending on the properties of the cluster surface, and R(t) is the radius of the cluster model at ambient temperature T_{amb}.

The first term in the right-hand part of equation (1) determines the energy received by bees at distance r owing to conduction by adjacent bees. The second summand characterizes the change in energy released by bees owing to metabolism. The function f(T) takes into account the heat production by individual bees through shivering and in the stationary state (without locomotion), as well as the heat released by bees at the cluster periphery in the state of superficial torpor.

According to Krogh's normal curve, the intensity of metabolism in insects at apparent rest rises with increasing temperature. The minimal heat production by a single bee is 1 to 1.5 mW (Owons, 1971; Heinrech, 1981 Janes et al., 2004). The temperature factor Q_{10} for metabolism (increase upon heating by 10 °C) is 2.4 (Eskov, 1995a). When the local temperature falls below 18 °C, the bee generates additional heat by shivering. The peripheral bees, having exhausted the resources in their honey stomachs, have to migrate into the heated zones of the cluster.

The experimental data available allow the maximal metabolism to be related to the resting metabolism, using a factor of 35 °C (Eskov, 1995a). Hence, the metabolism function of an individual bee can be presented as

$$f(x) = \begin{cases} 0.035e^{1.116(T-14)}, & when\ 8\ ^{\circ}\mathrm{C} \le T < 14\ ^{\circ}\mathrm{C} \\ 0.035, & when\ 14\ ^{\circ}\mathrm{C} \le T < 15\ ^{\circ}\mathrm{C} \\ 0.035e^{0.713(15-T)}, & when\ 15\ ^{\circ}\mathrm{C} \le T < 18\ ^{\circ}\mathrm{C} \\ 0.001 \cdot 2.4^{0.1(T-18)}, & when\ 18\ ^{\circ}\mathrm{C} \le T < 32\ ^{\circ}\mathrm{C} \end{cases} \tag{3}$$

The determination of the thermophysical parameters included in (1) is complicated by their dependency on the state of the bees, and they simultaneously play different roles in thermoregulation. It is impossible to integrate the heat capacity with the cluster volume because of the structural inhomogeneity and unordered bee migration. Therefore it is expedient to consider only the intervals at which the analyzed parameters may change.

The heat flux to the surface of the bee cluster is associated with the body heat capacity and depends on the water content in a body; the heat capacity of the air gaps between the bodies is insignificant. Assuming that the water content in the bee body is 65% and does not appreciably change through wintering (Eskov, 2003) and that the heat capacity of the hemolymph is close to that of water, which is 0.90–0.93 cal/(g °C), with increasing bee density their heat capacity changes roughly linearly from 0.6 to 0.8 cal/(g °C). The specific conductivity also increases with bee density from $7.6 \cdot 10^{-4}$ to $3.0 \cdot 10^{-3}$ W/(cm °C) (Omholt, 1987; Watmough, 1995); this is commensurate with the conductivity of dry air, $3.4 \cdot 10^{-4}$ W/($\mathrm{cm}^2 \cdot$ °C).

For heterogeneous systems with internal and surface heat sources (such as the bee clusters), the heat transfer coefficient is physically ambiguous, including in the general case the values of specific heat fluxes depending on conductivity, convection, and irradiation. The sum flux of heat from the cluster surface depends on the effective outer surface, i.e. on the area through which heat is removed. This is always smaller than the geometric surface area and depends substantially on the position of the cluster relative to the hive entrance. In addition, parameter a strongly depends on the water vapor percentage in the surface layer as well as on the heat loss by evaporation and respiration. Considering only the average heat loss from the cluster surface through convection, this parameter can be taken to range from $6 \cdot 10^{-4}$ to $3 \cdot 10^{-3}$ W/(cm^2 · °C) (Watmough, Camazine, 1995; Human et al., 2006).

The first equality in boundary conditions (2) stems from the condition that the temperature at the model center is maximal. The second equality is an expression of Newton's law taking account of heat irradiation.

In equation (1), the bees' density (r, t) depends on their motility and number and on the ambient temperature. In approximating the dependence of bee density on distance r (constant, linear, stepwise, etc.), the mean values were taken to be 2 bees/cm^3 in the center and 4-6 bees/cm^3 at the periphery (Eskov, Toboev, 2009c).

During wintering, the total number of bees N does not change significantly, so

$$N = 4\pi \int_{0}^{R(T_{\text{amb}},\, t)} r^2 \rho(r, t)\,dr \qquad (4)$$

can be regarded as constant. From this relationship, with a given density distribution, one can find the cluster radius $R(T_{amb}, t)$ to be a function of ambient temperature.

To solve equation (1) with boundary conditions (2), the numerical methods implemented in the software packages MatLab 7.02 and FemLab 3.01 were used; they allow the solutions to be examined with different values for the thermophysical parameters in the above-specified ranges. According to the calculations, the most essential parameters affecting the temperature profiles at the same ambient temperature are the heat's conductivity and irradiation. The smaller these coefficients are, the broader the high-temperature zone in the cluster is and the steeper the drop in the temperature curves is. Therefore, a reduction of the cluster size reflects a temperature rise in the core.

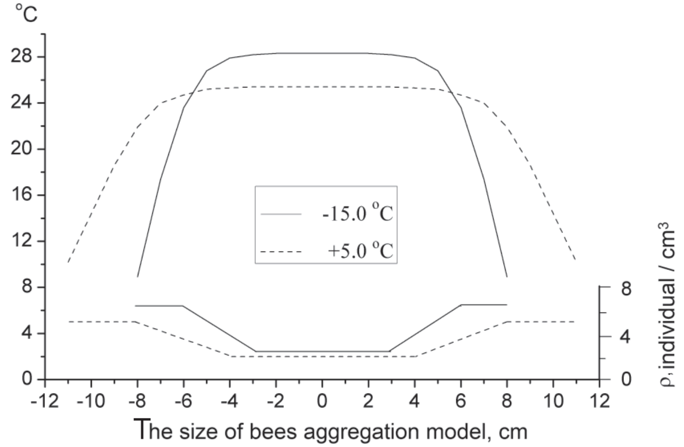

Fig. 23. Temperature profiles at a given bee distribution density (individuals/cm^3) inside the model parameters: N =18000, c – 0.8 cal/(g °C); $\lambda = 9.5 \cdot 10^{-3}$ W/(cm · °C); $\alpha = 8.8 \cdot 10^{-4}$ W/(cm^2 · °C).

With increasing conductivity and irradiation, the model also predicts a temperature rise in the center, but at the surface, the temperature drops below the preset limit even at maximal bee density (Fig. 23). Thus at $\alpha = 3.0 \cdot 10\text{-}3$ W/(cm^2 · °C), the model predicts a rapid cooling of the surface to sub-zero temperatures. The same happens upon an increase to $1.3 \cdot 10^{-2}$ W/(cm · °C). The positive temperature at the cluster surface, in this case, can be maintained only at the expense of additional heat production by the bees, but heat removal will also thereby increase.

The role of the second summand reflecting the individual metabolism becomes manifest at ambient temperatures below -5 °C. This is in quantitative agreement with the experimental result that locomotion does not suffice for efficient thermoregulation. These circumstances require the active production of heat by shivering (Soutwick, Maritz, 1971; Eskov, 1984, 1995a). The contribution of this process to thermoregulation is attenuated or maybe completely excluded at 2–9 °C, because in this temperature range the metabolism intensity is near its minimum, as judged by oxygen consumption (Eskov, 1995a).

At -12 °C, the production of heat by shivering becomes dominant in maintaining a positive cluster surface temperature. The function for metabolism (3) allows a conclusion that the main heat production load at low temperatures is borne by the bees immediately adjacent to those at the air-cooled surface. This permits the bees in superficial torpor to migrate to the warmed zone of the nest. The stimulus for migrating to the heat core harboring the food resources is obviously the hunger that commences upon depletion of the honey stomach (Eskov, 2016).

The model shows an inverse dependence of the thermal core temperature on ambient temperature (Fig. 24) and an increase in the core temperature with an increasing number of bees in the cluster. This is consistent with the data in the literature (Omholt, 1987; Eskov, 1995a; Watmough, Camazine, 1995) and with studies of thermal processes in cold-induced bee clusters, but which have not been explained heretofore (Omholt, 1987; Stabentheiner et al., 2003).

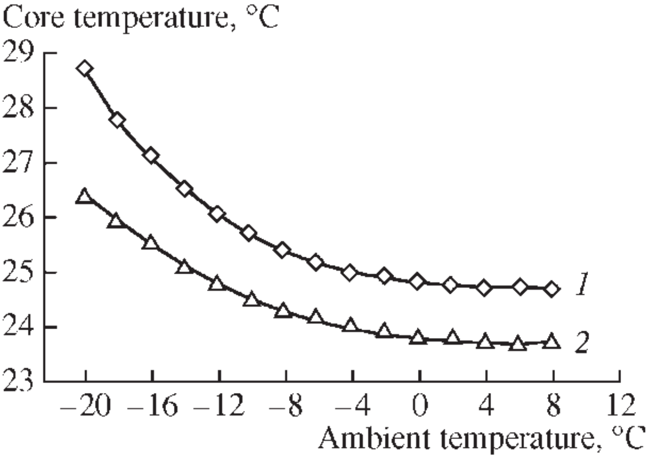

Fig. 24. Core temperature in model clusters of (1) 18,000 and (2) 12,000 bees as a function of ambient temperature; the model parameters were c – 0.8 cal/(g °C); $\lambda = 9.5 \cdot 10^{-3}$ W/(cm °C); and $\alpha = 8.8 \cdot 10^{-4}$ W/(cm^2 · °C).

Thus, a bee cluster represents a spatially open, demarcated biological system, the thermal processes in which can, with some reserve, be modeled using kinetic equations with preset initial and boundary conditions. However, since a priori such conditions are hard to relate to the real functioning of the biosystem, the modeling required an idealized notion of spherical symmetry in the cooled insect cluster. It must be emphasized that the thermophysical parameters of a model based on such an assumption cannot be physically unambiguous. To add the use of an equation of a new (2) for the boundary conditions of a Newtonian law is valid only if evaporation as a method of heat removal is completely absent.

Despite these limitations, the proposed model quantitatively reproduces the main features characteristic of the dynamics of heat processes in cold-induced insect clusters. In particular, it reliably predicts the core temperature rise in response to a deeper chilling of the periphery and the shape of the temperature profiles, as well as conditions simulating additional heat production and the thermoregulatory function of the bees adjacent to the outer layer. It reacts to ambient temperature variation by changing the cluster shape and density. Consolidation of the cluster associated with intense cooling demonstrates the lower insulation of the surface layer.

The considered theoretical model does not support the concept that an insect cluster is a superorganism where the dominant role in thermoregulation belongs to the portion of insects localized in the heat core (Owens, 1971; Stabentheiner et al., 2003). They have been supposed to react through enhanced activity and heat generation to elevated carbon dioxide levels resulting from compaction (Eskov, 1995a, 2016; Human et al., 2006). However, thermal imaging studies did not reveal the influence of bee heating in the core on bee heating at the periphery.

The relatively high body temperature of the outer bees is largely ensured by their heat generation and migration into the warmed zone. Thereby, their body temperature significantly exceeds that of maximal over-cooling and does not reach the chill coma threshold (Eskov, 2016). Migrations probably favor a reduction of excess moisture and carbon dioxide accumulating in the heat core.

The real enhancement of core heating in response to stronger cooling in the model corresponds to a decrease of the ambient temperature below the optimal range for a bee cluster (Fig. 23). At a certain relation between the thermal conductivity, heat transfer factor, and cluster radius, the temperature in the model’s center does not exceed 36 °C even during severe chilling at -40 °C. There can be no core overheating if the heat is produced by all bees to warm the surface layer or if heat production and compaction are performed by the bees in the sub-surface region to maintain the core temperature within a certain range. The increased heat emissions from the surface at a lowered ambient temperature and the ensuing stimulation of additional heat production by the sub-surface bees allow one to state that the thermoregulation mechanism corresponds to the principle of self-organization. However, the same bees, having come in during the course of clustering and subsequently migrated to different zones, perform dissimilar functions in thermoregulation. The greatest load during temperature regulation is carried by the bees at the cluster periphery because they are experiencing the unfavorable influence of chilling to the greatest extent and therefore must react to it more actively than the others.

Conclusion

Similarly to the other six species of true bees belonging to the genus *Apis*, the honey bee is a poikilotherm, which only partly fits the accepted division of the animals into poikilotherms and homoiotherms. Only the considerable dependence of the heat generation of the brood on air temperature completely corresponds to the principle of poikilothermy. This principle is partially modified in adult bees involved in various functions outside of their nests and does not fit the case of bee colonies at all, especially colonies that house a brood in their nest. Highly stable thermoregulation in the brooding zone of a bee nest allows this property of the honey bee to be ascribed to homeothermy.

Presumably, the acquisition of various adaptations providing for the protection of the honey bee colony from adverse environmental effects has imposed some restrictions on the ecological valence of its embryos, larvae, and pupae. Presumably, the use of efficient ethological mechanisms for stabilizing the brood temperature was accompanied by the acquisition of pronounced stenothermy. On the other hand, due to the thermoregulation of the brood, the honey bee occupies a unique position in the class of terrestrial insects with respect to the stability of its ontogenetic transformations and insignificant variations in morphometric traits.

The regulation of brood temperature by worker bees involves the implementation of a complex instinct that was developed in conjunction with deepening social relationships, which enhanced the transformation of the bee colony into an evolving biological unit that is individually subject to natural selection and other elementary factors of evolution. However, it is likely that not all adult bees are able to provide heating, or at least not at any time, since only some of them that are in the brooding cells generate heat in certain biological situations.

The odorous secretion that enters through the porous cell caps is of paramount importance in implementing the instinct of heating the sealed brood, which is why bees do not regulate the temperature in the wax-sealed cells filled with stored feed that do not display this specific feature. Once a bee has the motivation to heat the brood, it starts to generate heat that responds to the signal, which is the relatively low temperature of the cell surface. The feedback between an adult and a developing bee is established according to its response to heat radiation, which increases in living pupae when they are heated. The absence of this response allows bees to detect dead pupae in wax-sealed cells. An increase in carbon dioxide evolution by a heated pupa can serve as an additional signal that enables communication with a living individual. A combined temperature sensor additionally detects carbon dioxide, and it can enhance the stimulatory effect of the pupal heat radiation.

Despite the fact that the bee colony can survive long-term cooling to -35 and even -45 °C, each of its members displays low tolerance to cooling (they can withstand cooling to no lower than -16 to -17 °C). Similarly to other poikilotherms, bees respond to cooling with a chill coma, in which they can retain viability for several days. Viability during a chill coma depends on the MOT; the value of solitary living insects varies depending on the age and season. The specific feature of MOT for bees is its relatively fast changes during overwintering, namely, its increase during periods of starvation and decrease when feeding on carbohydrates and hypoxia. This considerably influences the bees of overwintering colonies that are localized to the most heated nest area, its heat center. That is why the cold tolerance of bees increases when staying in the heat center and decreases when they are localized in the nest periphery.

The adaptation of the honey bee to cold climates has not involved the physiological mechanisms that enable the long-term and deep arrest of vital processes, which is typical of many solitary-living insect species. Presumably, it is not biologically purposeful for honey bees to diapause during adverse periods of their lives, since the bees themselves concentrate in large numbers in limited spaces of various dwellings; their stored feed needs protection from predators and robbers. Therefore, bees have developed adaptations that make it possible for at least a part of their colony to retain an active state, which is necessary and sufficient for their own safety and the protection of their feed reserves.

To a considerable degree, the large reserves of carbohydrate feed that accumulate in the nest during the spring-summer season and its economic use enable bees to withstand long overwintering. The arrest of the queen bee’s reproductive function before overwintering and the location of the majority of bees beyond the heat center enhance the economical use of energy resources. The state of the bees that form the periphery of their consolidated swarm (except for the area above the heat center) may approach or reach a chill coma, which reduces the metabolic activity by several dozen times.

The processes of heat generation, accumulation, and loss in the consolidated bee swarms stimulated by cooling are interconnected and depend on the external temperature. This temperature also influences the aggregation activity, the volume occupied by insects, and energy expenditures for their vital activities. A multitude of random factors determine the localizations of individuals in the formed swarm. However, each bee responds to the temperature change in a specific manner that corresponds to its hereditary program.

Bees respond to cooling by compaction, and the corresponding decrease in the swarm surface consequently causes a decrease in heat loss, since the total heat flow depends on the area through which heat is lost. The heat inside the swarm can be produced by bee bodies and in part by convection through the air gaps between them. The surface heat loss is provided by free convection and radiation.

The heat circulation index, which is the ratio of external to internal temperature gradients, is mainly determined by the intensity of heat flows from the inner zones to the periphery of the bee swarm, the structure and shape of which changes according to the variations of external temperature; its decrease has a maximal effect on the heat circulation index at the bottom surface of the swarm and a minimal effect at the upper surface. An increase in the circulation index towards the bottom surface is associated with a decrease of its heat insulation. That is why the bees that form the lower part of the swarm are affected by cooling to a maximal degree, which induces them to actively respond to a temperature decrease by the activation of thermogenesis.

A circadian behavioral program associated with the random periodicity of changes in temperature and other external factors acquired in the phylogenesis of this species allows for an increase in adaptation to their typical ranges of variations. However, the conditions for the biologically purposeful implementation of the hereditary program for synchronizing the behavior with cyclic circadian patterns are limited by the fact that the ranges of their intensity vary considerably during the annual cycle of the bee colony's life. Therefore, the strict programming of their behavior is biologically unreasonable, which determines the need to combine a circadian program with individual experience. The use of this experience by bees manifests as an increasing independence of the thermoregulatory processes inside the nest from the beginning of overwintering to its end. The desynchronization of the circadian rhythms of heat generation and loss in the case of the considerable external heating or cooling of the bee colony leads to the desynchronization of the circadian system.

The certain periodicity of thermoregulatory activity, which bees retain under constant external conditions, is characteristic to different degrees of both poikilotherms and homoiotherms. The large variations in temperature in poikilotherms are associated with the changes in locomotor activity. With a decrease in locomotion, the body temperature of poikilotherms approaches the external temperature (Schmidt-Nielsen, 1982; Eskov, 1992). The effect of locomotor activity on the body temperature of homoiotherms is weaker as compared with poikilotherms (Ivanov, 1972; Eskov, 1992). However, their weight is important; a decrease in weight reinforces the dependence of body heating on external temperature, which is also characteristic of honey bee colonies (Eskov, 1995a).

In a resting state, endothermal rhythmic temperature changes in homoiotherms are maintained by numerous hierarchically connected oscillators that differ in their ability to generate autonomous oscillations. In vertebrates, the suprachiasmatic nuclei of specialized cells of the central nervous system can serve as leading oscillators. Note that thermoeffectors can act to regulate body temperature and operate in relatively independent loops without a single control system.

Similarly to a poikilotherm, a consolidated bee swarm lacks any centralized mechanism for temperature control and regulation. The interaction of bees in a swarm is provided according to the principle of decentralized control, which implies that numerous subsystems (individuals) make up the system and act using only limited local information obtained from thermoreceptors. Note that there are no information contacts or interactions between the aggregated bees localized in different parts of the swarm, which are widely used in the spring-summer season to protect the nest, harvest feed, and so on (Eskov, 1979).

The coordinating role in the thermoregulatory system for the overall swarm belongs to the integral temperature, which corresponds to the average level of heat content. This is suggested by the absence of the distinct stability of the heat pattern inside the swarm. The temperature gradients in the swarm, which characterize the heat content, are maintained in the optimum temperature range at a relatively constant level. The temperature decreases approximately 1.5-fold from the heat center to the upper swarm boundary and approximately threefold to the lower boundary. Presumably, the heat content stabilizes the interaction of different heat profiles of the swarm, where bee localization depends on the vector of temperature effects (cooling or heating). The activity of thermogenesis changes accordingly, as well as the selection of the direction for migration.

A more or less pronounced convergent similarity is evident between the set of cold adaptations of consolidated bee swarms and homoiotherms. This allows aggregated bees to be used to model heat generation and dissipation by homoiotherms. Similar to a homoiotherm, the swarm of aggregated insects has a region of high-temperature stabilization, i.e. a heat center. However, in the absence of a brood, heating varies in a range that, in certain biological situations, exceeds 10 °C. The core body temperature in one of the most highly organized homoiotherms, humans, varies within a range of 2 °C. The cooling limit of the periphery of the bee swarm in the optimal temperature range for overwintering is limited by the temperature that induces their chill coma. Therefore, it is necessary and sufficient for bees to maintain an approximately threefold difference in the temperature of the heat center and the swarm's periphery, which is the most affected by cooling. As for humans, under comfortable temperature conditions, the core body temperature (37 °C) elevates the temperature of surface tissues by approximately 10%. However, at an external temperature of 0–3 °C, the human skin temperature can be cooled to 22 °C. The human (Konietzny, Hensel, 1975) and bee (Heran, 1952; Lacher, 1964) thermoreceptors respond to a temperature change at a rate of several tenths of a degree per second. The sensory summation of the information from many receptors allows one to control temperature change by several hundredths of a degree per second (Prosser, Brown, 1967), which is most likely utilized in the thermoregulatory systems of both mammals and bees.

CHAPTER THREE

ADAPTATIONS TO GAS AIR COMPOSITION

In the planetary boundary layer, the air is a mixture of gases, water vapor, and dust. The largest volume fractions in the air are occupied by nitrogen and oxygen – 78.09 and 20.95%, respectively. Carbon dioxide (CO_2) accounts for 0.02–0.04%. The CO_2 content in a bee dwelling can reach several percent.

Water vapor, forming a homogeneous mixture with the air, can pass from a liquid to a solid phase (ice). Air saturation with water vapor varies widely depending on humidity, temperature and atmospheric pressure. The maximum amount of water vapor that can be in a unit volume of air increases with increasing temperature. In a state of saturation, if the temperature and pressure do not change, the water vapor is in neutral equilibrium with the water surface. When the temperature decreases, water vapor condenses, and when it rises, the air becomes saturated with water vapor due to evaporation.

Air saturation with dust occurs as a result of natural atmospheric processes and under the influence of anthropogenic activity. Dust can have a mineral, organic and cosmic nature. Mineral dust is formed as a result of the destruction of rocks, volcanic eruptions, forest, peat, and other fires. Organic dust is represented by aeroplankton (bacteria, pollen, fungal spores, etc.), products of fermentation and decomposition.

Variability of the CO_2 and O_2 content in the bee dwelling

The honey bee exhibits a high tolerance to hypoxia, which is connected with its adaptation to life in shelters and especially with the ability to aggregate in response to cooling (Hess, 1926; Budel, 1955; Free, 1958; Michener, 1974; Eskov, 1995a). Bee aggregation around initially heated areas in the nesting space reduces heat loss but prevents the removal of CO_2 released during respiration from the bee clusters (Heinrich, 1985; Eskov, 1992, 2003). During long cold snaps, the CO_2 concentration in the clusters of bees may exceed the content of this gas in the ambient air by hundreds of times (Eskov, 1995a).

Tolerance to hypoxia has allowed bees to occupy a wide range of habitats due to their settlement in shelters. However, hypoxia affects the physiological state of adult and developing bees in cold and mild climates. For this reason, the bees have ethnological means adapted to counteract the adverse effects on them of high concentrations of CO_2.

Oxygen is consumed and CO_2 is released during the respiration of adults and developing bees. The content of these gases in a bee house depends on the efficiency of its aeration, the ecological situation and the physiological state of the bees.

The role of the brood. At different stages of the brood's development, from the egg to the pupa, oxygen consumption varies widely. The main contribution to CO_2 saturation in the hive is made by the brood (pupa and larvae of the middle and older ages). The O_2 consumption by larvae and pupae changes during their development. The O_2 consumption by larvae per unit weight decreases in the period from the beginning to the end of the larval stage. This trend continues in the period of transformation of larvae to prepupae and pupae. The O_2 consumption by pupae decreases by middle age and increases by the end of their development.

Regardless of the age and the weight of developing bees, O_2 consumption increases with temperature from the lower to the upper limits of the vital range. The response of larvae to the same temperature change is less pronounced than the response of pupae. In particular, when the temperature increases from 30 to 40 °C, the oxygen consumption by 1–3-day-old larvae increases, on average, by 1.6 times, and by pupae – 1.9 times.

Adult individuals. In the spring-summer period, the adult population of a bee dwelling is mainly represented by working bees and drones. Oxygen consumption by adults in the nest depends mainly on their activity and the frequency of abdominal respiratory movements, during which the air sacs are compressed and expanded. The entry of the air into the tracheal system depends on the length of the period during which the spiracles are in the open state. It also affects the direction of air circulation in the tracheal system. Most often, air enters the abdominal region from the thoracic region. In cases of the saturation of the air with CO_2, inspiration through the abdominal spiracles and expulsion through the thoracic spiracles is possible (Bailey, 1954).

Depending on the locomotion activity, the bee's abdomen can perform 40–150 respiratory movements per minute. In the processes of chill coma and anesthesia, the frequency of abdominal contractions decreases beyond the specified minimum value. In a state of chill coma or anesthesia, the abdomen remains motionless. During the flight, breathing is intensified by the deformation of the thoracic region with the muscles of indirect action. The transverse muscles contract with a frequency of about 250 Hz, and in the longitudinal muscles, it is twice as high (Eskov, 1969). Airbags of the thoracic region are subjected to these deformations, and as a result, oxygen consumption increases by approximately 50 times (Hocking, 1953).

The maximum change in the intensity of respiration occurs under the influence of the lowering of the temperature from the optimal level to that which stimulates a cold chill coma. Young bees, compared to old ones, react to this change in temperature to a rather smaller extent. Younger bees consume the greatest amount of oxygen in the brood zone of the nest, where the temperature is consistently maintained at about 35 °C. At this temperature, 3-day-old bees consume oxygen at an average quantity of 11 mm^3 per min. Their consumption decreases by 1.6 times at 40 °C and by 85 times at 0 °C. In bees of the middle and older age groups, the highest oxygen consumption is 56.6 mm^3/min, which occurs at 30 °C. At 0 °C it decreases by 218 times, and at 40 °C it decreases by 1.7 times.

In a range that does not go beyond the limits of the inhibition of locomotion, queens react by intensifying respiration in response to an increase in temperature, like bees. Unlike younger bees, the queens (up to 10 days old) consume the greatest amount of oxygen at 40 °C, averaging 25.4 mm^3/min. With a decrease in temperature of 10 and 20 °C, oxygen consumption decreases by 1.7 and 1.9 times. In 1–3-year-old ovulating queens, oxygen consumption in the indicated conditions increases approximately twofold.

In the spring-summer period, drones are usually localized on the periphery of the nest, where the temperature is 5–10 °C lower than in the brood zone. At 25 °C, drones consume a relatively large amount of oxygen, amounting to 66 mm^3/min. When the temperature rises to 30 and 40 °C, their oxygen consumption decreases by a factor of 1.5 and 2.6, respectively.

The respiratory quotient value depends on temperature. In worker bees, in the range of temperatures stimulating the chill coma, it increases. At 10 °C, the value of the respiratory quotient is 1.3; at 0 °C, the respiratory quotient is 1.6; at 15–35 °C – ≈ 1; and at 40 and 50 °C – 0.85 and 0.81, respectively. In queens under the age of 10 days, the respiratory quotient at 30 °C is 0.9 ± 0.05, whereas in 1–2-year-old queens in the period of high reproductive activity, the respiratory quotient is 0.7. In drones, in the range of optimal temperatures, the respiratory quotient is approximately 1.

According to the values of the respiratory index, in worker bees, carbohydrate metabolism dominates in the range of 15-35 °C. A significant use of fats and proteins occurs under the influence of increasing the temperature to 40 °C and above. A high respiratory coefficient at temperatures stimulating the chill coma is apparently caused by the release of a certain amount of CO_2 from the body's tissues without oxygen consumption. CO_2 can also be formed as a result of the synthesis of fats from carbohydrates. The value of the respiratory coefficient, which is close to unity at the optimum temperature, is caused by the consumption of carbohydrate food by young queens and drones. In contrast, an intensive consumption of protein feed by ovulating queens is reflected in a lowering respiratory quotient.

Adult bees, unlike queens and drones, are involved in the aeration of the nesting space along with oxygen consumption. This is achieved by the flapping of the wings of bees (ventilating bees), the number and activity of which depends on the concentration of CO_2 saturating the nesting space (Eskov, 1983). A significant limitation of air exchange between the breeding space and the external environment is associated with the cold aggregation of bees. The density of bee clusters and the content of CO_2 in them increases with a decreasing external temperature. A particularly high CO_2 content, reaching 6–8%, is maintained in the centers of bee colony clusters (Eskov, 1995a).

Seasonal and daily dynamics of CO_2 and O_2 in bee dwellings. During the bee colony's annual life cycle, the content of CO_2 and O_2 in its nest changes significantly. The greatest change is related to the concentration of CO_2. Its minimum and maximum values can differ by more than 100 times. A relatively low content of CO_2 and high O_2 is typical for the spring-summer period. However, at this time, the concentration of CO_2 in the nest may vary by more than 20 times, depending on the environmental situation.

Developing bees and drones. During the periods of high bee activity, the CO_2 concentration in the brood zones of the nest can decrease to 0.05–0.15%, and during cold snaps, it can increase to 1.6–2.2% and, less frequently, to 2.5–3.0%. In the warm summer season, when bees replenish their feed reserves, the CO_2 concentration in the central part of the inter-cell space averages 0.35 ± 0.12%, and at its periphery – 0.27 ± 0.11%, while the O_2 concentration averages 19.9 ± 0.2% and 20.3 ± 0.3%, respectively.

In the zones of temperate and cold climates, at the end of the summer to the beginning of the autumn, the concentration of CO_2 begins to increase, and O_2 – to decrease. This trend becomes steady with the onset of cold weather. With daily temperature fluctuations from 0 to 10 °C, CO_2 concentration in the center of the nest is set in the range from 0.9 to 2.5%, and from the side facing the entrance door – from 0.4 to 1.2%, while the O_2 concentrations, respectively, vary between 10 to 16% and 15 to 19%.

In the second half of wintering, after the completion of the reproductive diapause, which is accompanied by the activation of bees, there is a decrease in the concentration of CO_2 and an increase in O_2. In January-February, in the center of the nests of colonies wintering indoors at +0.5 ± 3.5 °C, the CO_2 content is maintained at an average level of 2.4%, from the entrance side – 0.7%, above the center – 0.96%, below it – 0.81%, and from the back wall of the hive – 0.62%. The concentration of O_2 in the center of the nest rises on average to 8.9%, and from the side of the bee entrance to 17.2%.

The highest concentration of CO_2 and the lowest O_2 content are set at the end of the autumn and the first half of the winter. At this time, in the central part of the nest, the CO_2 concentration can be maintained at a level of 3.5–4.5% for a long time (several weeks). Less commonly, the CO_2 concentration can reach and be maintained at 6–7% for several hours. This is accompanied by a decrease in O_2 to 6–8%. For colonies wintering in closed temperature-controlled rooms at 0 ± 5 °C, the CO_2 content at the periphery of the nest in the inter-cell spaces at the entrance side is usually in the range of 0.9 to 1.6%, and O_2 – 16 to 14%.

With regard to the environmental factors on the composition of the gaseous environment in the bee's nest, the greatest influence on them is exerted by the external temperature (Fig. 25). Local gradients of CO_2 and O_2 depend on it in the zones occupied by developing worker bees, drones and queen bees. In the space between adjacent cells (these cells are occupied by developing worker bees), the concentration of CO_2 at the same temperature has relatively small differences, but strongly depends on its daily and seasonal fluctuations. Thus, during periods of summer cooling, when the external temperature is about 5 °C, the concentration of CO_2 in the center of the nest occupied by the brood is maintained at 1.3 ± 0.1%, above it (at the upper limit of the localization of the brood) – 1.2 ± 0.1% and below it – 1.0 ± 0.1%. When the external temperature rises to the level optimal for the bee colony, the CO_2 concentration in these zones decreases on average to 0.5 ± 0.07%, and at 35 °C it approaches the external one, exceeding it by 3–4 times.

The ratio between the number of adult bees and larvae significantly affects the amount of CO_2 in the nest. If at the end of May this ratio was in the range of 1.1 to 2.2, then the correlation coefficient between the number of larvae and CO_2 concentration was 0.75 ± 0.15. The increase in the ratio between the number of adult bees and larvae to 4.4–10 by the end of July–beginning of August was accompanied by a change in the correlation to a negative one between the number of larvae and CO_2 concentration, which was set at the level of -0.54 ± 0.25. Obviously, the larvae with a need for a large amount of oxygen stimulate adult bees to aerate the nest. However, a relatively small number of bees at the end of spring could not fully satisfy the oxygen needs of the larvae by aerating the nest.

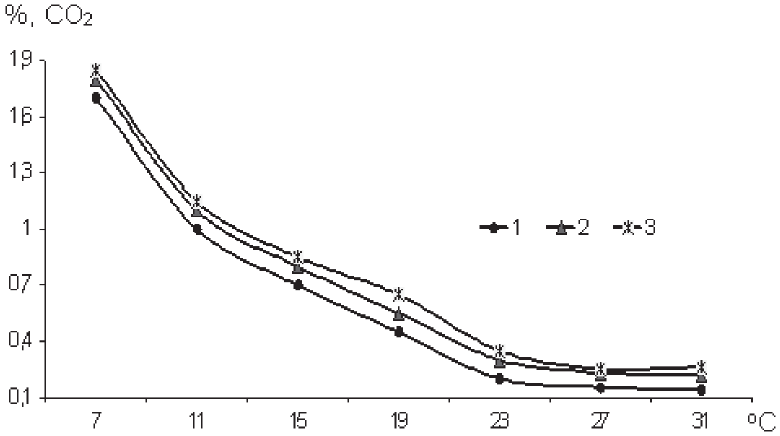

Fig. 25. The influence of the external temperature on the CO_2 concentration in different areas of developing drones' and bees' localization: 1 – from the side of the lower bee entrance; 2 – in the center of the nest; 3 – above the center.

Developing queens. The concentration of CO_2 in the queen cells depends mainly on the physiological state of the bee colonies, the number of bees in them, and the external temperature. The gas composition in the zones of queen cells' localization largely coincides with its composition in developing bees and drones. However, the placement of the queen cells in the nest and the specific attitude towards them by the worker bees are reflected in local gradients of CO_2 concentration.

There are some differences in the concentration of CO_2 in the queen cells, depending on their localization in the nest (Fig. 26). For example, in the bottom corner of the comb (from the entrance side), at 8–30 °C, the CO_2 concentration at the queen cell averages 0.44%, at the center – 0.53%, and at the top at the hive's back wall – 0.62%.

Under the influence of an increase in temperature from the level preventing flight from the hive to the upper limit of the optimal range or beyond, the greatest decrease in the CO_2 content occurs in the queen cells, which are located in the lower part of the nest from the entrance side. In this zone, the increase in temperature from 8 to 30 °C leads to a decrease in the concentration of CO_2 on average by 6.6 times, in the center – 4.6 times, and above – 5.7 times.

No changes in the CO_2 concentration were detected in the developing queen after the transformation of the larvae into prepupae and pupae. During the entire period of the development of queens from larva to imago, with fluctuations in the external temperature from 9 to 26 °C, the average daily values of CO_2 concentration in the queen cells located in the center of the nest did not exceed 0.51–0.75%.

Queenless colonies. Bees of queenless colonies with ovulating working bees are characterized by increased aggressiveness and locomotor activity. This is due to some excess in their nests, relative to the average rate, in the concentration of CO_2. In particular, in developing drones (the offspring of ovulating bees) localized in the lower part of inter-cell spaces, when the external temperature fluctuates from 10 to 30 °C, the CO_2 concentration averages 0.71%, in the center – 0.82%, and in similar areas of the colony with queens – 0.67 and 0.70%, respectively. With a decrease in external temperature, these differences increase.

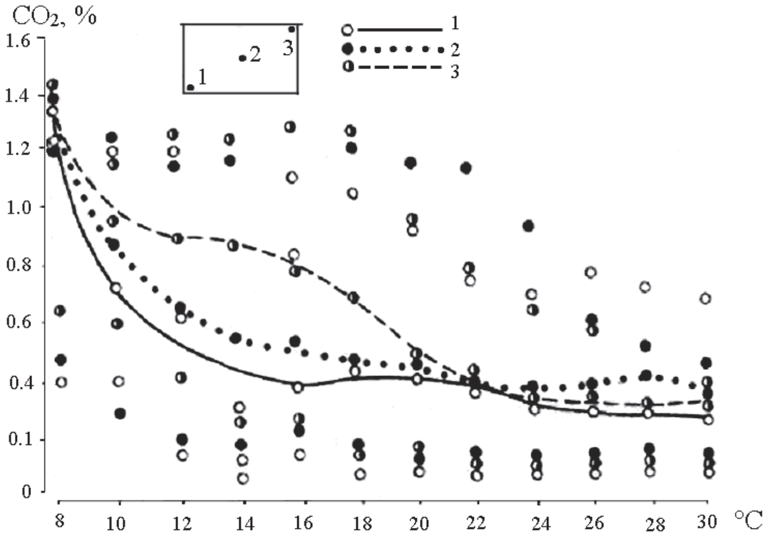

Fig. 26. The effect of external temperature on the CO_2 content of the queen cells located from the side of the bee entrance (1), in the center (2) and above, from the side opposite to the inlet (3).

The introduction of honeycombs to a queenless colony with developing worker bees slightly normalizes its condition. However, this does not have a significant effect on the change in the intra-nesting concentration of CO_2. At the same time, the introduction of a brood of ovulating bees into the nest of a colony with a queen effects a certain increase in CO_2 concentration. Apparently, this reflects the negative attitude of the bees living with the queens towards the offspring of ovulating working individuals from the queenless colony.

The effect of hypoxia on the bees' development

The honey bee has adapted to the high saturation of CO_2 in the nesting space, which ensures the survival of the species in cold climates. But this does not exclude the direct and prolonged effect of high CO_2 concentrations on the physiological state, development and viability of bees. Under natural conditions of the life and development of bees in which there is a high variability of CO_2, it is not possible to establish the concentrations that have teratogenic and lethal effects. Therefore, studies were undertaken in which the bees developed in natural conditions and at a given temperature with an artificially controlled gas composition for the air (Eskov, 2016; Eskov et al., 2014a, 2014b). The natural conditions include the content of bee colonies in typical hives without the use of agents that affect the state of the gas atmosphere in them. Under artificial conditions, brood and adult bees removed from hives were kept in thermostats at a given temperature, relative humidity and concentration of chemically pure carbon dioxide.

Natural habitat. Under natural conditions, an approximately five-fold increase in the CO_2 concentration relative to the mean value (on average, from 0.45 to 2.5%) in the area of worker bees' location only slightly affected their morphometric parameters and physiological state. A marked decrease was observed only in the length of the proboscis, which strongly depends on changes in the developing conditions. Slight changes were also observed in the fat and nitrogen content in the body of bees. At the above-mentioned degree of air saturation with CO_2, the proboscis' length and the nitrogen content decreased by 3.4 and 0.3%, respectively, whereas the fat content increased by 1.8%. The size of the exoskeleton and the body weight did not change significantly. In the bees that developed in a 0.45% CO_2 atmosphere, the width of the fourth tergite was, on average, 4.84 mm and the body weight was 102 mg, whereas at 2.5% CO_2, these parameters were 4.80 mm and 103.4 mg, respectively.

An increase in the CO_2 concentration from 0.5 to 2.5% throughout the entire larval stage of queens caused only a slight reduction in the proboscis' length (on average, 0.5%). This decrease was accompanied by a decrease in the number of egg tubules and the body weight by 6.6 and 2.9%, respectively. However, it caused no changes in the size of the abdominal tergites (Eskov, Toroptsev, 1978).

The morphometric parameters of worker bees and queens did not change under the influence of increasing concentrations of CO_2 from 0.1 to 3% in the course of their development from the prepupal to the adult stage. However, an increase in the CO_2 content to 6% caused a reduction in the width of the fourth tergite of worker bees and queens by 1.3 and 3%, respectively. In addition, the duration of the pupal stage of queens increased by 5.5 ± 1.5 h.

Artificial (specified) conditions. Under natural conditions, from the egg stage to the completion of the development of the pupae, the brood is often subjected to intensive elimination. Adult bees detect and eat or remove sick and weakened individuals from the nest (Eskov, 1995a, 1995b). In the spring-summer period, the intensity of elimination of the brood largely depends on the ecological situation and, especially, on the productivity of the forage area and the spring temperature. Optimal conditions for the condition of bee colonies and brood development are provided for by the productivity of the forage area, which satisfies or slightly exceeds the daily need of the bee colony for its carbohydrate and protein feed.

The temperature at which the bee colonies spend the minimum amount of energy on life support is limited to 22–28 °C. A high productivity of the feeding area is unfavorable for brood because the majority of the adult population of bee colonies in such cases is engaged in the delivery of nectar and its processing into honey. Often in such situations, the bees fill the cells occupied by eggs and larvae of a younger age. A low productivity of the feed area, which does not meet the daily need of bee colonies in their carbohydrate feed, is also unfavorable for brood. In such conditions, the trophic supply of the larvae does not satisfy the needs necessary for their normal development (Eskov, 1992, 1995a).

Metabolic activity. The larvae of worker bees at the optimum temperature for their development (33–35 °C) respond to a decrease in the content of O_2 in the air from 19.5 to $6 \pm 0.4\%$ with a decrease in O_2 consumption by a factor of 13.9 ($P > 0.999$). The respiratory quotient values in this case increased, on average, from 1.45 ± 0.04 to 2.94 ± 0.26. A temperature decrease to 29 °C at 19.5 and 17% saturation of the air with O_2 caused a 1.2- and 2.1-fold decrease in the metabolic activity, respectively. Under the same hypoxic conditions at 37.5 °C, the O_2 consumption increased by 1.1 and 1.4 times, respectively (Table 5).

Table 5. Dependence of the respiratory quotient and O_2 consumption of larvae (29 ± 8 mg) and old pupae of worker bees on the gas atmosphere's composition and temperature.

Temperature (°C)	Oxygen content (%)	Respiratory quotient	Oxygen consumption (cm^3/g·h)
	Larvae		
29 ± 0.5	19.5 ± 0.9	1.45 ± 0.04	1.28 ± 0.08
	16.8 ± 2.1	2.16 ± 0.16	0.33 ± 0.04
33.5 ± 0.5	19.5 ± 0.09	1.43 ± 0.11	1.53 ± 0.12
	16.8 ± 2.1	2.31 ± 0.18	0.69 ± 0.06
	13.7 ± 1.4	2.87 ± 0.23	0.38 ± 0.04
	6.1 ± 0.4	2.94 ± 0.26	0.11 ± 0.02
37.5 ± 0.5	19.5 ± 0.09	1.39 ± 0.07	1.73 ± 0.14
	16.8 ± 2.1	2.23 ± 0.16	0.94 ± 0.12
	Pupae		
29 ± 0.5	19.5 ± 0.9	1.05 ± 0.03	0.98 ± 0.09
	16.1 ± 2.1	1.61 ± 0.11	0.56 ± 0.06
33.5 ± 0.5	19.5 ± 0.09	0.96 ± 0.08	1.43 ± 0.08
	16.1 ± 2.1	1.59 ± 0.10	0.86 ± 0.07
	12.7 ± 1.4	2.05 ± 0.18	0.23 ± 0.03
37.5 ± 0.5	19.5 ± 0.09	1.09 ± 0.04	1.72 ± 0.11
	16.1 ± 2.1	1.64 ± 0.09	0.97 ± 0.08

Hypoxia had a long-term inhibitory effect on metabolic activity. For example, the O_2 consumption of the bee larvae that were first incubated at 33.5 °C for 40–60 min at 8–14% O_2 concentration and then transferred to a normal air medium was 0.37 ± 0.08 cm^3/(h·g), which was almost five times smaller than the physiological norm (Eskov, Eskova, 2011).

The consumption of O_2 by pupae, similarly to larvae, decreases when its content in the air decreases (Table 5). At the optimum temperature, a decrease in the O_2 concentration by 19.5–12.7% is accompanied by a decrease in the O_2 consumption by a factor of 6.2. As the temperature decreased to the lower boundary of the vital range (29 °C), the O_2 consumption was reduced. At the lower boundary of the vital range, a reduction in the O_2 concentration relative to its normal content in the atmosphere by 1.4–5.8% causes a 1.5-fold decrease in the O_2 consumption, whereas at the upper (37 °C) boundary, the O_2 consumption increased by 1.1–1.2 times. A decrease in the O_2 concentration in the gas atmosphere from 19.5 to 16.1 and 12.7% leads to an increase in the respiratory quotient value in pupae from 0.96 ± 0.08 to 1.59 ± 0.11 and 2.05 ± 0.18, respectively.

Viability and development anomalies. The effect of hypoxia on developing bees during the incubation period up to the imago stage depends on the conditions in which the brood developed from egg to prepupa (cell sealing) in the nests of their colonies. All the bees that developed to the prepupal stage in thermostats (control) under conditions of an optimum temperature and natural gas atmosphere environment lived to the imago.

Development at the optimum temperature, but in a gaseous medium with a high concentration of CO_2, was reflected in a decrease in the viability of the bees. In the case of the incubation of brood at 5% CO_2 concentration, the death of bees did not exceed 0.1%. It increased significantly with an increase in the concentration of this gas on average to 10 and 15%, to 14.4 ± 2.5 and 24.8 ± 4.1%, respectively.

Anomalies of development, expressed in the underdevelopment of wings and the proboscis, were found only in those bees that developed in a gaseous environment with a CO_2 content exceeding 5%. Wings were absent (there were only their rudiments or undeveloped wing plates) in 5.1 ± 1.4% of bees that developed at a 10% concentration of CO_2, and in 14 ± 2.4% when the content of this gas was 15%. The same bees had an underdeveloped proboscis. The minimum length for the former was 4.5 mm, for the latter – 4.2 mm, and their average values were 5.47 ± 0.327 mm and 4.65 ± 0.401 mm, respectively.

Bees that developed up to the prepupa stage in colonies with an external temperature not exceeding 20 °C, at a time when the plants in the feed area did not emit nectar, were distinguished by a reduced tolerance to hypoxia (Eskov et al., 2013a, 2013b). In the case of development at the prepupa and pupae stages at 5 ± 1% CO_2 concentration, their death was at the level of 1%. 68 ± 2.7% did not survive until the imago stage if the brood was in a gaseous environment with 10% CO_2 content for the first six days. If, during the first six days, the bees developed in a natural air environment and then at 10% CO_2 concentration, 59 ± 2.6% of the developed individuals died. All the bees died in the case of the incubation of the brood at 10–12% CO_2 concentration during the entire period of the development of the bees in sealed cells (12 days at 34 °C).

Hypoxia (10–12% CO_2 concentration) in the initial or final phases of the development of sealed brood had a different teratogenic effect. The wings and proboscis were underdeveloped in 33 ± 11.7% of bees that reached the stage of imago under the conditions of a specified CO_2 content during the first 6 days. There were 2.6 times ($P \geq 0.99$) fewer such bees if they were subjected to hypoxia during the last six days of development in sealed cells.

Body weight and water content. The development of bees under hypoxic conditions is reflected in the increase in the weight of different regions of their body and of the entire body. In the bees that developed in a hive to the prepupal stage and then to the adult stage under optimum conditions, the weight of the cephalic region was 11 ± 0.27 mg. The incubation of the brood at the optimum temperature caused an increase in the head weight, on average, of 3.9% at 5% CO_2, 5.9% at 10% CO_2, and 6.5% at 15% CO_2. Similarly, the weight of the thoracic regions increased by 1.1, 3, and 3.4%, and the weight of the abdominal regions increased by 0.5, 4, and 4.3%, respectively. For bees that developed to the prepupal stage under unfavorable conditions, the weight of their body and its regions was smaller, on average, by 5.3%. The changes in these parameters caused by hypoxia did not differ significantly from the changes observed in the bees that initially developed under optimum conditions.

The increase in the weight of different regions of the body of bees under the influence of hypoxia was accompanied by an increase in the water content in them. A slight increase was detected in the bees that developed at 5% CO_2. The increase in the water content was particularly significant in the ventral regions of the bodies of bees that developed at 10 and 15% CO_2 concentrations. The water content in them increased by an average of 17 and 19.3%, respectively. Under these conditions, the water content increased by 3.3 and 4.1%, respectively, in the cephalic regions and by 1.1 and 1.3%, respectively, in the thoracic regions. In the generation of bees that developed in thermostats with free access of air, the water content in the cephalic regions was 71.6 ± 0.69%, in the thoracic regions – 72.1 ± 0.52%, and in the abdominal regions – 83.8 ± 0.61%.

Morphometric parameters. For bees that had no marked morphological abnormalities, the lengths of the proboscis and wings and the number of claws decreased as the concentration of CO_2 in the gas atmosphere increased. When it increased from 0.1 to 5, 10, and 15%, the length of the proboscis decreased by 4.5, 6.4, and 7.5% ($P > 0.99$). For bees that developed at 0.1% CO_2 concentration, the proboscis' length was 5.61 ± 0.084 mm.

A high concentration of CO_2, in which the bees developed for some time at the pupal stage, had a significant effect on the length of the proboscis. Under the influence of the development of CO_2 in a 15% atmosphere for three days, the proboscis' length decreased on average to 5.5 mm, and in a 30% atmosphere – to 4.5 mm. The range of variability of the proboscis' length in bees that developed at 15% CO_2 content varied from 4.2 to 6.3 mm, and at 30% – from 3.8 to 4.9 mm (Fig. 27).

The sublethal levels of hypoxia caused a decrease in the length of the proboscis by 25–30%. Of the structures of the proboscis, the uvula underwent the greatest shortening. Its length decreased by 1.3–1.6 times.

The length of the left and right wings (Fig. 27) and the number of claws on the hind wings changed in different ways under the influence of elevated CO_2 concentrations. In the natural gas atmosphere, the length of the left and right forewings was 9.74 ± 0.036 mm and 9.56 ± 0.021 mm, respectively. When the concentration of CO_2 increased from 0.1 to 5, 10, and 15%, the length of the right wing decreased by 2.7, 5.4, and 9.7%, and the length of the left wing decreased by 2.6, 3.3, and 7.3%, respectively. Under these conditions, the length of the left hind wing decreased by 5.3, 6.8, and 11.5%, respectively (versus 7.02 ± 0.027 mm at 0.1% CO_2), and the length of the right hind wing decreased by 2.7, 3.4, and 7%, respectively (versus 6.76 ± 0.44 mm at 0.1% CO_2) ($P > 0.99$).

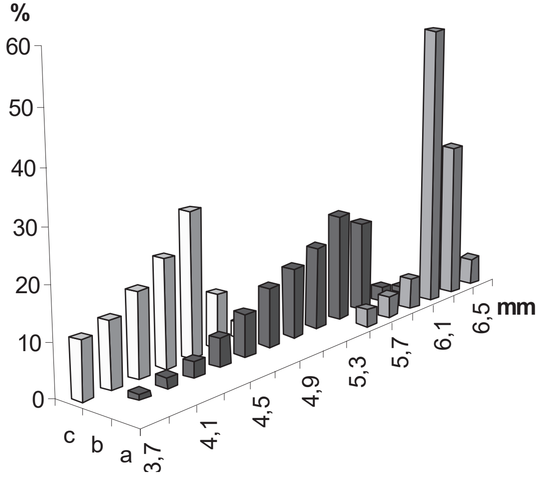

Fig. 27. Representation of bees (%), differing by length of proboscis; bees at the pupal stage developed under normal conditions (a) or for three days in a 15% (b) or 30% (c) CO_2 atmosphere.

Similarly to the changes in the size of the left and right hind wings, the number of claws on them showed a similar decreasing trend and an increased asymmetry as the CO_2 concentration increased (Fig. 28b). At the specified increased values of CO_2 concentration, the number of claws decreased by 0.9, 2.1, and 5.1%, respectively, on the left wing, and by 0.7, 1.5, and 3.6%, respectively, on the right wing ($P \geq 0.9$) (versus 21.74 ± 0.245 and 21.56 ± 0.261, respectively, at 0.1% CO_2).

The decrease in the size of wings and the length of the proboscis of the bees that developed under hypoxic conditions was accompanied by an increase in the limits of their variability range. In the bees that developed under free access of air, the length of the anterior wings ranged from 9.3 to 10 mm, whereas in those that developed at 5 and 10% CO_2, the increased variability was accompanied by a decrease in the minimum values to 8.6 and 8.2 mm, respectively. In the bees that developed in a gas atmosphere containing 15% CO_2, the variability of the length of wings slightly decreased as a result of reducing the maximum value to 9.4 mm. The proportion of bees with such wings was, on average, 5.6%. The minimum value of the length of wings (8 mm) was observed in 3.3% of bees (Fig. 28).

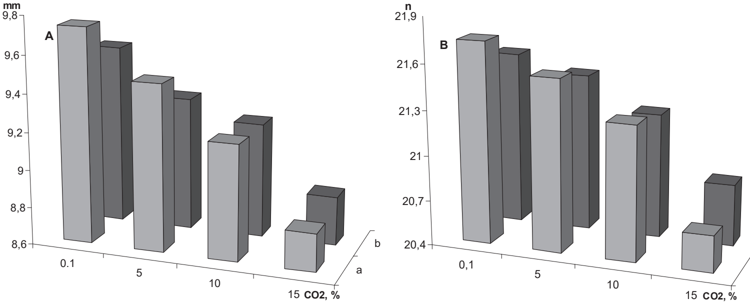

Fig. 28. The length of the front wings (A) and the number of claws on the rear wings (B) of bees that developed from the prepupa stage to imago in a gas atmosphere differing in CO_2 concentration (a – left wings, b – right wings).

The variability of the size of the hind wings differed from the variability of the front. In the case of development in a natural air environment, the range of variation in the length of the hind wings was limited to 6.6–7.4 mm. In the bees that developed at a 5% CO_2 concentration, the length of the hind wings varied from 6.1 to 7.3 mm, at 10%, from 6.1 to 7 mm, and at 15%, from 5.5 to 6.8 mm.

The increase in the CO_2 concentration during the bees' development from the prepupa to the imago stage was accompanied by a specific variation in the crop's length. Normally, it varied from 4.7 to 6.3 mm (in approximately 70% of bees, it ranged between 5.5–6.3 mm). In the bees that developed in the medium with 5% CO_2 content, the proboscis' length varied from 4.2 to 6.2 mm (in 65% of bees it ranged within 4.6–5.3 mm). The shortest proboscis was found in some bees that developed at 10% CO_2. The distribution of the proboscis lengths of these bees, ranging from 4 to 6.2 mm, had two peaks in the frequency of occurrence at 4.3–5.1 mm (~50%) and 5.5–5.8 mm (~30%). A similar distribution pattern with respect to the proboscis length was characteristic of the bees that developed at 15% CO_2 concentration (Fig. 29).

For bees that developed to the prepupal stage under unfavorable conditions, after the completion of their development in thermostats with free access of air, the length of the proboscis was 5.01 ± 0.08 mm, the length of the left forewing was 9.31 ± 0.03 mm, the length of the right forewing was 9.39 ± 0.04 mm, the length of the left hind wing was 6.69 ± 0.03 mm, and the length of the right hind wing was 6.70 ± 0.02 mm. The number of claws on them was 20.1 ± 0.19 and 20.2 ± 0.21, respectively.

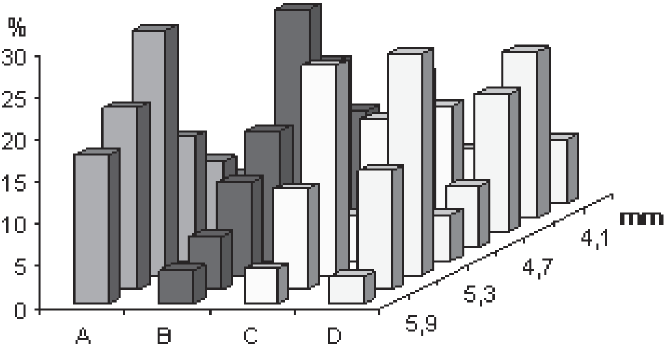

Fig. 29. Distribution by the length of the proboscis (%) of the bees that developed at the pupal stage in natural conditions (A) with (B) 5, (C) 10 and (D) 15% CO_2 concentration.

The representation of bees that survived to the stage of imago under conditions of deep hypoxia changed significantly. In bees that developed at 5 and 10% CO_2 concentrations, there was an increase in the range of variability along the length of the wings and a decrease in the minimum values to 8.2–8.6 mm. The maximum length of the wings of bees developing in a 15% CO_2 atmosphere was 9.4 mm, but the representation of bees with such wings did not exceed 6%. The minimum value of the wings (a length of 8 mm) occurred only in 3.3% of cases. In the bees that developed with 0.1% CO_2 content in the air, the variability of the wing length was limited to 9.3–10 mm (Fig. 30).

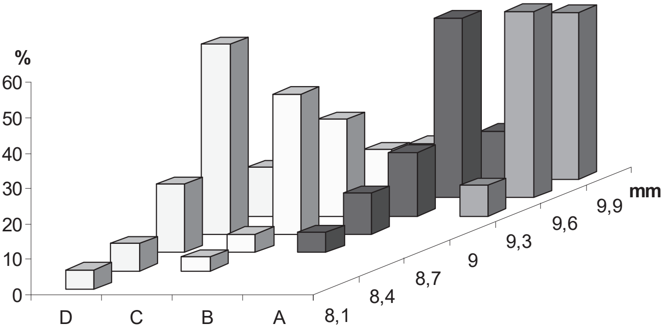

Fig. 30. Distribution of variation (%) in the wing length of bees that developed from the prepupal to the adult stage in (A) a natural gas environment and at (B) 5, (C) 10, and (D) 15% CO_2 concentration.

Depending on the favorable conditions, the length of the proboscis after the completion of development was 6.1 ± 0.08 mm, the front left wings – 9.31 ± 0.03 mm, the right wings – 9.39 ± 0.04 mm, and the back left wings – 6.69 ± 0.03 mm and 6.70 ± 0.02 mm. The number of hooks on them was 20.1 ± 0.19 and 20.2 ± 0.21, respectively.

Incubation at 10% CO_2 in the first six days led to a reduction in the length of the forewings and hind wings, on average, by 16 and 15.1%, respectively, and the number of claws on them decreased by 3.2%. For bees that developed under these conditions in the last six days, the length of the left forewing and hind wing was reduced by 4.8 and 13.7%, respectively, and the number of claws on them decreased by 2.5%. The proboscis' length in the first and second groups decreased by 8 and 6.4%, respectively ($P > 0.99$). In the bees that developed at 5 ± 1% CO_2 during the entire period from the prepupal to adult stage, the length of the proboscis and the left forewing and hind wing decreased by 6, 6.7, and 5.4%, respectively, and the number of claws on them decreased by 1.5%.

Under the influence of elevated levels of CO_2, the asymmetry of wings changed, which was manifested in a greater decrease in the length of the left wings with respect to the right ones. As a result, the initially greater length of the left wings relative to the right ones was reversed. For bees that developed at 15% CO_2 concentration, the right wings became longer than the left ones.

Hypothermia under hypoxic conditions

The effect of hypoxia on the viability and development of bees was traced in the sealed brood that developed in the hives of colonies until the end of the larval stage and unsealing of the honeycomb cells. Honeycombs with broods of the same age withdrawn from the hive were cooled for a certain time in a natural environment or in an atmosphere of chemically pure CO_2. After the completion of cooling, the broods were incubated under a natural air environment at 34 °C. The broods that were not subjected to cooling were simultaneously incubated with free access of air.

Viability. It was found that the cooling of broods to 0 °C for 1.5 h in a natural air environment led to the elimination of 15.1 ± 3.1% of bees at the end of development under optimal conditions. When the brood was subjected to cooling for 3 h, 20.4 ± 7.6% of bees did not survive to the adult stage. Hypoxia at 0 °C intensified the elimination of bees. Cooling the broods for 1.5 h at a 100% replacement of air with CO_2 led to the elimination of 26.6 ± 4.9% of the developing individuals. All developing bees incubated under such conditions for 3 h died at different pupal stages.

The incubation of bees at 25 °C for 3 h in a natural air or a 100% CO_2 atmosphere did not affect their mortality rate. Among the bees that developed at this temperature for 20 h with free access to air and under hypoxic conditions, the losses were 0.4 ± 0.2 and 40 ± 5.2%, respectively.

The body mass. The cooling and hypoxia of the broods caused a decrease in the body weight of the bees that reached the adult stage. The weight of the cephalic, thoracic, and abdominal regions of the bees incubated under optimal conditions was 12.43 ± 0.21, 37.95 ± 0.58, and 57.55 ± 1.81 mg, respectively. The cooling to 0 °C for 1.5 h with free access of air reduced these body regions by 5, 6.2, and 8.9%, respectively; under hypoxic conditions, these parameters decreased by 9.7, 7.2, and 16% ($P \geq 0.95$).

The incubation of the broods at 25 °C for 20 h caused significant changes in the weight of the cephalic regions. Under free access of air and under hypoxic conditions their weight decreased, on average, by 10.2 and 11%, respectively. The weight of the abdominal regions decreased by 7.6 and 18%, respectively, and the weight of the thoracic regions was reduced by 1.2 and 2.7%, respectively.

Morphometric features. The cooling of the broods differentially changed the size and symmetry of wings. In the bees that were incubated at 0 °C for 1.5 h, the length of the left and right forewings decreased, on average, by 2.5 and 1.8%, respectively, and the length of the left and right hind wings decreased by 3.3 and 2.3%, respectively ($P \geq 0.99$). The incubation at 25 °C for 20 h led to the shortening of the left and right forewings by 3 and 2.2%, respectively, and the left and right hind wings by 4.3 and 4%, respectively ($P > 0.99$). Under the influence of hypoxia, these differences in the bees that developed for the specified time at 0 °C increased by 5.6, 4.5, 4, and 2.9%, respectively, relative to the control; at 25 °C, they increased by 5.1, 3.7, 5.4, and 4.5%, respectively ($P > 0.99$). The reduction in the length of the hind wings was accompanied by changes in the number of claws on them.

The cooling and hypoxia of the broods had the greatest impact on the length of the proboscis. Under the influence of cooling to 0 °C for 1.5 h with free access of air, the length of the proboscis decreased by 16.1%. Hypoxia enhances the effect of cooling; as a result, the length of the proboscis decreased by 19.1%. Similar changes in the length of the proboscis were observed after incubation for 20 h with free access of air or in a CO_2 atmosphere; in this case, the length of the proboscis decreased by 16.4 and 19.6%, respectively ($P > 0.999$).

Thus, the cooling of bees at the pupal stage leads to an increase in their elimination before the achievement of the adult stage. Hypoxia enhances the elimination of the cooling's efficiency and the effect on the mass of body parts, as well as morphometric signs. The specific effect of cooling and hypoxia on the morphometric characteristics of bees is reflected in a change in the proportions of the left and right wings. As their length decreases under the influence of cooling and hypoxia, the probability of right-sided dominance in this trait increases.

Response of adult bees to hypoxia

The CO_2 content in a bee nest is always higher and the O_2 content is lower than in the atmospheric surface layer. Therefore, when staying in the hive, bees are always more or less exposed to hypoxia, the severity of which depends on the seasonal dynamics of CO_2 and O_2. The greatest oxygen starvation of bees is experienced in the fall and winter, the least – in the spring-summer periods of the annual life cycle (Eskov, 1992, 2003). Depending on the biological situation and the saturation of the CO_2 gas environment, the bees react by activating or are anesthetized and numbed.

Ethological and physiological effects of hypoxia. Elevated CO_2 levels in the hive space stimulate bees to aerate the nest by flapping their wings. The number of flapping wings of the bees (ventilating bees) increases when the CO_2 concentration exceeds a certain threshold level (Seeley, 1974), which changes in the course of the annual life cycle of bees. In summer and winter, the activity of ventilating bees is stimulated by an increase in the CO_2 concentration in the periphery of the hive by 1 and 3%, respectively. Ventilating bees respond to an increase in the CO_2 content by increasing the frequency of wing flaps (Eskov, 1992).

The subthreshold CO_2 concentrations, which do not stimulate the aerating activity, may have an inhibitory effect on bees, which has been shown for bee colonies wintering under temperature-controlled conditions (Eskov, 1995a). In the colonies wintering at 0 ± 3 °C, the correlation coefficient between food intake and mean CO_2 concentrations in the above hive space was -0.56 ± 0.13. The minimum and maximum food intake in different bee colonies in winter was 5.8 and 17.3 kg, respectively, and the CO_2 concentration in them varied from 0.6 to 2.5%.

The increase in temperature led to a decrease in the CO_2 concentration in the hive. Accordingly, the effect of hypoxia on food intake was reduced. When the wintering bee colonies were kept at 7 ± 2 °C, a correlation between the food intake and the CO_2 concentration was absent (correlation coefficient – $r = 0.1 \pm 0.21$). The CO_2 concentration in the above-nest space of different colonies ranged from 0.4 to 1.8%, and the mean daily food intake varied from 22 to 56 g.

When bee colonies wintered at elevated CO_2 concentrations, their potential involvement in the growth of the brood decreased. In this case, the temperature of wintering was a major factor. In the colonies that wintered at 0 ± 3 °C, the correlation coefficient between the CO_2 concentration in the above-nest space and the number of cells of the broods grown in early spring by overwintered bees was -0.46 ± 0.16, whereas for bees that wintered at 7 ± 2 °C, the r was -0.62 ± 0.19. This difference is apparently due to the fact that an increase in temperature enhances the effect of hypoxia, which consists of the reduction of the potential possibility of their participation in feeding the brood.

The content of CO_2 in the nest of wintering bees affects the consumption of reserve nutrients by them. The r between the CO_2 concentration in the above-hive space and the nitrogen and fat content in the body of bees at the end of the wintering period was -0.58 ± 0.17 and -0.48 ± 0.16, respectively (Eskov, 1995a, 2003). These data reflect the effect of hypoxia on the physiological senescence of bees, which limited their participation in the feeding broods after wintering.

Physiological effects of anesthesia. Carbon dioxide has a narcotizing effect, which is expressed in the total or partial immobilization of bees. Bees are anesthetized when the content of CO_2 in gas atmospheres exceeds its content in the surface layer of the atmosphere by approximately three orders of magnitude. Two-day-old bees were anesthetized at 30 °C within 80 min in a 30% CO_2 atmosphere (Deyme, Belgue-Deyme, 1977) and within 15–30 s in a 100% CO_2 atmosphere (Eskov, 1995a).

Activation and elimination of anesthetized bees. The time required to activate the anesthetized bees, other things being equal, depends on the duration of anesthesia and the age of the bees (Eskov et al., 2013b). After incubation in a 100% CO_2 atmosphere at 22–24 °C for 30 min, young and old bees of the summer generation were activated, on average, in 14.7 ± 0.9 and 18.9 ± 1.1 min, respectively. When the duration of hypoxia increased to 120 min, the time required for the activation of young and old bees increased to 33.3 ± 1.2 and 41.1 ± 2.3 min, respectively.

The time required for the activation and death of anesthetized bees largely depends on the temperature. The effect of hypoxia at a temperature promoting the chill coma of bees was not significant. In particular, under free access of air, the mortality of medium-age bees after incubation at 0 °C for 6 ± 0.5 h was 10 ± 2%; for 55 ± 4 h, 50 ± 6 %; and for 67 ± 5 h, 100%. In the case of incubation in a 100% CO_2 atmosphere, approximately 10% of bees died within 3 ± 0.5 h; 50%, within 43 ± 5 h; and 100%, within 63 ± 4 h.

Temperature fluctuations in spring and summer had different effects on the viability of bees under hypoxic conditions. Incubation in 100% CO_2 at 25 °C (optimum temperature for adults) led to the elimination of approximately 50% of bees after 4.4 ± 0.4 h; at 35 °C, after 1.7 ± 0.2 h; and at 45 °C, after 0.7 ± 0.1 h. Under these conditions, all bees died within 7, 5.5 and 2 h, respectively.

The time required to activate the torpid and/or anesthetized bees depends on the duration of their life in these states. When the duration of chill coma at 0 °C increased from 2 to 60 h, the time required for the activation of bees increased from 11 ± 2 to 86 ± 9 min in natural cold air and from 21 ± 3 to 190 ± 12 min under hypoxic conditions.

Bees that were anesthetized at 25 °C for 1 h were activated within 22.3 ± 3.1 min in a natural air atmosphere. When anesthesia continued for 3 h, the time required for their activation increased to 51.9 ± 4.3 min. As the temperature increased, the activation time of anesthetized bees also increased. The bees that were anesthetized at 35 °C for 1 and 3 h were activated within 67.4 ± 5.6 and 133.5 ± 8.7 min. The bees that were anesthetized at 45 °C for 30 min and 1 h were activated within 21 ± 1.6 and 182 ± 12.8 min.

The time required for activating the anesthetized bees depends on their physiological state, which for worker bees strongly depends on their age (Eskov, 1995a). After a 30 min exposure to CO_2, the physiologically young bees, whose contribution to feeding the brood was negligible or absent, were activated within 14.7 ± 0.9 min, whereas the old bees were activated within 18.9 ± 1.1. After a 120-min incubation, the activation times increased to 33.3 ± 1.1 and 41.4 ± 2.3 min, respectively. After repeated anesthesia sessions, the time required to activate the bees increased. The activation time of young and old bees increased from the first to the fourth 30-min anesthesia sessions by a factor of 1.52 and 1.31, respectively (Eskov et al., 2013a).

The life expectancy of bees exposed to anesthesia. The exposure of adult bees to hypoxia reduced their lifespan. The latter decreased insignificantly when the bees were exposed to 100% CO_2 for no longer than 30 s. As the duration of anesthesia increased, the lifespan of bees steadily declined. In particular, the lifespan of bees that were not subjected to anesthesia was, on average, 42.4 days; after anesthesia in a CO_2 atmosphere for 5, 10, and 20 min, it was reduced by 14, 29, and 43%, respectively (Austin, 1955; Skowronek, Jaycox, 1974; Rindfleisch, 1977; Tustain, Faulke, 1979).

The effect of large anesthesia exposures was traced in the bees kept in entomological cages at 26–27 °C. These bees consumed 50% sucrose, which favors survival in isolation (Eskov, 1995a). As a result, the bees that did not undergo hypoxia lived for 34 ± 4.8 days. The life expectancy of bees that survived anesthesia for 60 minutes at 25 °C was 14 ± 1.9 days, and for 120 minutes – 8 ± 1.6 days.

Hypoxia intensified the eliminating effect of chilling to the chill coma. The bees that survived chilling to 0 °C for 7 h under free access of air were eliminated within 10 ± 1.5 days, whereas the anesthetized bees were eliminated within 7 ± 0.9 days. An increase in the duration of the chill coma in a natural gas atmosphere to 48 h caused a reduction in the lifespan of bees to 3.2 ± 0.5 days, and the lifespan of bees that were subjected to both chilling and hypoxia decreased to 2.1 ± 0.4 days.

Change in the body mass and its water content. Anesthesia with CO_2 led to changes in the body weight of bees and the water content in them. These changes were temperature-dependent. Anesthesia in the temperature range varying from the temperature maintained in the central area of the nest to the temperature that caused chill coma had a similar but not identical effect on the body weight dynamics of bees. After incubation for 3 h at 25 °C, the weight of the cephalic, thoracic, and abdominal regions of the anesthetized bees decreased, on average, by 11.5, 6, and 6.1%, respectively; after incubation at 35 °C, these parameters decreased by 13.5, 7.4, and 7.6%, respectively ($P > 0.99$) (Table 6). Incubation for the same time under free access of air caused no significant changes in the weight of the body and its regions.

When the bees stayed in the chill coma in the natural air at 0 °C for 3 h, the weight of the cephalic, thoracic, and abdominal regions decreased by 1.5, 1.8, and 1.3%, respectively; for 7 h, by 3.8, 2.6, and 2%, respectively; for 24 h, by 6.0, 6.7, and 6.4%, respectively; and for 48 h, by 16.4, 9.5, and 15.5%, respectively ($P > 0.99$).

Anesthesia in CO_2 reduced the effect of chill coma. In the bees that were anesthetized at 0 °C for 3 h, the weight of the cephalic, thoracic, and abdominal regions decreased by 0.8, 1, and 0.3%, respectively; for 7 h, by 2.3, 2.1, and 0.7%, respectively; for 24 h, by 5.3, 4.1, and 2.4%, respectively; and for 48 h, by 15.2, 8.2, and 11.5%, respectively. The initial weight of the cephalic, thoracic, and abdominal regions of bees was, on average, 13.2 ± 0.22, 38.8 ± 0.54, and 68.6 ± 0.59 mg.

Table 6. Changes of mass and water content in different parts of bees under the influence of anesthesia with carbon dioxide for 3 hours at 25 °C (B) and 35 °C (C); A – initial state.

Conditions	Head	Chest	Abdomen
	M ± m lim	M ± m lim	M ± m lim
Mass (mg)			
A	14.8 ± 0.18 13-17	40.3 ± 0.41 36-45	72.1 ± 1.9 48-98
B	13.1 ± 0.2 10-16	37.9 ± 0.4 32-41	67.7 ± 2.1 56-92
C	12.8 ± 0.2 10-16	37.3 ± 0.5 33-44	66.6 ± 2.6 39-88
	Water content (%)		
A	70.3 ± 0.51 66-79	67.1 ± 0.44 63-73	76.6 ± 0.72 69-88
B	66.3 ± 0.59 62-75	64.7 ± 0.41 62-71	68.7 ± 1.42 66-85
C	65.6 ± 0.68 61-73	64.3 ± 0.4 61-70	67.9 ± 1.21 65-82

The body weight dynamics under the influence of hypoxia depended on the age of the bees. After anesthesia for 60 min, the weight of the cephalic, thoracic, and abdominal regions of the young bees (12.1 ± 0.3, 37.9 ± 0.5, and 52.8 ± 1.8 mg, respectively) decreased by 10.7, 4.7, and 7.4%, respectively. In the old bees these changes for the same period accounted for 5.8, 2.1, and 5.2%, respectively, of the initial weight of the cephalic, thoracic, and abdominal regions of 8.6 ± 0.2, 33.4 ± 0.9, and 56.7 ± 1.9 mg, respectively.

The decrease in body weight in all considered situations was accompanied by a decrease in the water content. After incubation for 3 h at 25 °C, the water content in the cephalic, thoracic, and abdominal regions decreased by 4.0, 2.4, and 7.9%, respectively; at 35 °C, it decreased by 4.7, 2.8, and 8.7%, respectively (Table 6).

In the torpid bees in a natural air environment and a 100% CO_2 atmosphere, changes in the water content were similar or the same. In the former, water losses in the cephalic, thoracic, and abdominal regions reached 3.7, 4.3, and 4%, respectively, for 24 h and 8.2, 5.6, and 6.3%, respectively, for 48 h. Under hypoxic conditions, these changes accounted for 3.6, 3.9, and 3.8%, respectively, for 24 h and 7.8, 5.1, and 5.9%, respectively, for 48 h. Initially, the water content in the cephalic, thoracic, and abdominal regions was 73.7 ± 0.59, 67.7 ± 0.49, and 85.1 ± 0.67%, respectively.

Condition of ovarioles, hypopharyngeal and wax glands. Under the influence of anesthesia (for up to 3 days) for 15 minutes, the development of young bees' ovarioles is delayed and their fertility decreases if they are kept in colonies without queens. This does not occur if the bees are kept without queens for 4–6 days before anesthesia, as this stimulates the development of ovarioles in them. It has also been found that development was inhibited in the bees that did not undergo anesthesia but were kept together with those who were anesthetized in a 100% atmosphere of CO_2 three times for 15 minutes (Harris, Harbo, 1990).

Hypoxia affects the development of hypopharyngeal glands in different ways, depending on the duration of its action and the concentration of CO_2. The containment of bees for 11 ± 4 days at 10% CO_2 concentration is reflected in a 1.9-fold increase in the size of their ovarioles (Eskov, 1995a). But their development is inhibited by a 20-minute anesthesia of young bees in 100% CO_2. Under the influence of anesthesia, by the age of 14–21 days, the hypopharyngeal size is 45 ± 2% less than that of non-narcotic patients (Skowronek, Jaycox, 1974).

The anesthesia of young bees also retards the development of wax glands and the fatty body associated with their functioning (Skowronek, Jaycox, 1974). Normally, by the average age of spring-summer generations of bees, the height of wax glands in them reaches 50–60 microns (maximum – 80 microns). Their height in bees that were anesthetized was 35 ± 5 μm (maximum – 62 μm). The body fat underdevelopment of these bees was 6–30%.

Age-related variation of polyethism of worker bees. The functional differentiation of working bees in a bee colony is not related to their external differences but depends on their age (Langstroth, 1909; Phillips, 1930). Young worker bees at the age of 1–12 days are mainly engaged in feeding the larvae, which is associated with the active functioning of the hypopharyngeal glands secreting royal jelly. When the bees are 11–13 days old, they begin to develop wax glands, which allow them to participate actively in the construction of combs. Bees usually begin to engage in the delivery of food from 17-21 days of age. In some extreme situations, for example, in the case of the elimination of bees of the older age groups, young bees begin to perform functions characteristic of old bees (Gaydak, 1969; Eskov, 1992). Bees anesthetized at the beginning of the imaginal stage started participating in food delivery at an earlier age. The bees that were anesthetized for 10 and 20 min began to participate actively in food delivery 1 or 2 days earlier (Skowronek, Jaycox, 1974).

The effect of hypoxia on the variation in the flight activity of bees decreased with increasing the age at which they were subjected to anesthesia (Skowronek, Jaycox, 1974). After anesthesia at the age of 10 days, the flight activity of forager bees first increased and then (after approximately 5 days) sharply decreased and became even lower than that of the unanesthetized bees. The anesthesia of 20-day-old bees did not stimulate their flight activity. Conversely, it drastically decreased 2–3 days after anesthesia. After that time, all bees completely ceased to deliver pollen, whereas approximately one-third of the anesthetized bees of the same age returned to the hive with pollen.

Development and attractiveness of queens. The anesthesia of queens at the beginning of the imaginal stage accelerates their development. They start ovipositing earlier than unanesthetized bees (Mackensen, 1947). However, long-term anesthesia suppresses the motivation of queens to fly for mating. For example, three 10-min exposures to 100% CO_2 with 3-day intervals were sufficient to prevent mating. After the completion of the development of ovarioles, such queens laid only unfertilized eggs (Moeller, 1976).

A single 10- or 20-min anesthesia of 2-day-old queens reduced the number of their mating flights by approximately 1.5 times, which began and ended later than the flights of the anesthetized queens. After the completion of mating flights, the sperm count in the spermatheca receptor of the anesthetized queens was, on average, 13% smaller than that in the unanesthetized queens. In the breeding season, the proportion of anesthetized queens that remained unfertilized was 8% greater than the unanesthetized queens (Skowronek, 1976).

Under the influence of anesthesia, the attractiveness of queens decreased. The number of bees attracted by the queen after 20 min of anesthesia had decreased by 1.2 times on day 4, 1.9 times on day 11, and 1.5 times on day 18 (Skowronek, 1976). This phenomenon can apparently be explained by the inhibitory effect of anesthesia on the secretion of attractants.

Anesthesia decreased the potential ability of queens to produce worker bees. This is due to the fact that the sperm count in the sperm receptor of the queens, subjected to 20-min anesthesia, decreased in the next 1–2 years of life more rapidly than in the unanesthetized queens. In addition, the anesthesia of queens reduced their lifespan (Skowronek, 1979). Apparently, this was due to the intensification of the physiological aging of queens.

Moisture content of the bee house and water content in the bee body

The water vapor content in a bee hive is subject to the influence of a wide range of biotic and abiotic factors. Depending on the physiological state of the bee colonies and the weather conditions, water vapor is unevenly distributed within the bee dwelling and the nesting space occupied by the bees.

The content of water vapor in the air is characterized by various indicators (specific, absolute and relative humidity of the air, dew point, partial pressure of water vapor, lack of moisture), but the physiological perception of humidity is most satisfied by relative humidity.

Humidity in the bee house. The saturation of the nesting space with water vapor largely depends on the temperature. The moisture content of the air entering the nest during its exchange with the external environment is associated with the temperature. At the time of the spring-summer season, when the internal nesting temperature exceeds the external one by no less than 10 °C, its fluctuations have the opposite effect on changes in the internal nest humidity.

Periodic fluctuations in the relative humidity of the air in the bees' nest are clearly visible in the spring-summer period (Fig. 31). For example, in the nest of a colony containing about 25 thousand adult individuals, during the day at 17–25 °C, the relative humidity in different zones of the brood's placement was maintained at 48–84%, and at night at 7–10 °C it decreased to 30–63%. The humidity of the outside air during these days changed, respectively, from 40 to 60% and from 90 to 100%.

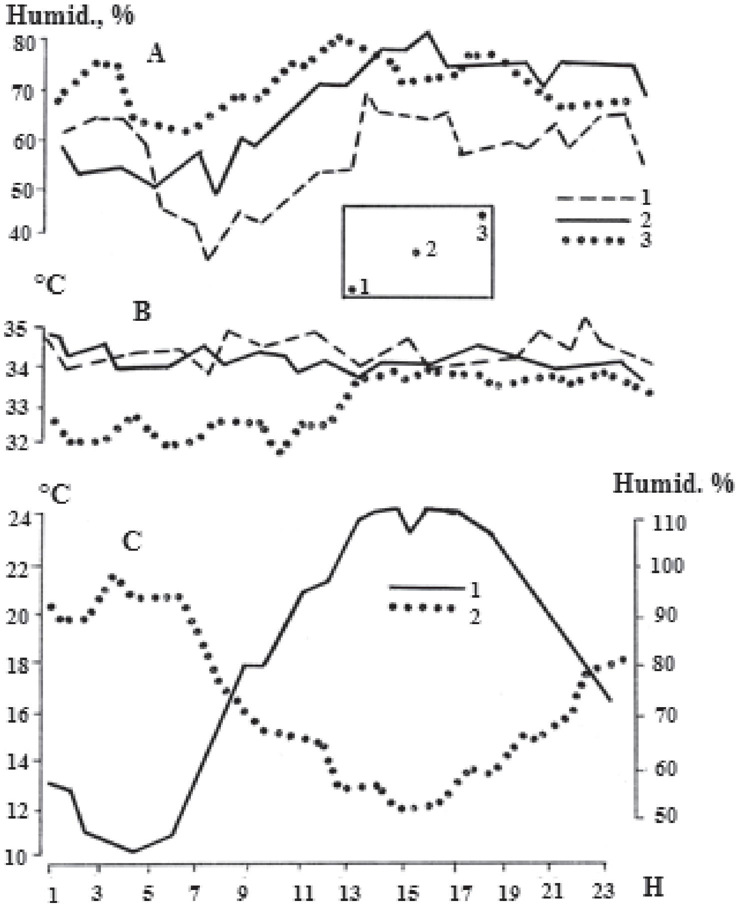

Fig. 31. Interrelations of daily changes in temperature and humidity: A – intra-nesting air humidity in different zones of the nest (1–3) between cells occupied by the brood; B – temperature in the same zones; C – external temperature (1) and air humidity (2).

The distribution of water vapor in different zones of the nest depends on fluctuations in external temperature and humidity. The greatest variability in humidity occurs in the nesting zone located on the side of the inlet. The relative humidity of the air in the nest of the colony containing about 25 thousand bees was 41 ± 3% in this zone in the morning at 10–11 °C. By the middle of the day, when the external temperature reached 23 ± 3 °C, the air humidity in the influx zone reached 70–75%. In smaller limits, the humidity in the central and upper zones of the nest varied – from 50–53 to 78–83% and from 62–65 and 73–81%, respectively. The relative humidity of the outside air at night approached 100%, and during the day it dropped to 50–60%.

In the autumn-winter period, the reproductive diapauses of the queens significantly modify the behavior and physiological state of the bees. They experience their deepest and most prolonged hemodynamic at this time. The aggregation of the bees at the thermal center limits the air exchange between the space they occupy and the external environment, but the nesting space, when free of bees, is strongly influenced by the external temperature and air humidity.

During the wintering period, the greatest differences in the humidity of the air are the zones in which the bees are located and the free spaces outside of them. Within these zones, the water vapor content is unevenly distributed and may vary significantly depending on the environmental situation. In the inter-cell spaces occupied by bees, the water vapor content reaches a maximum in the zone with a relatively high temperature (thermal center). In zones free of bees, the highest humidity is maintained above the upper limit of their localization, and the lowest – under the bottom. Some leveling of the water vapor content in spaces not occupied by bees occurs during thaws due to the entry of warm air saturated with water vapor into the dwelling.

Moisture receptors. The honey bees' receptors that react to changes in the relative humidity of the air are represented by the whole-conical, ampoule-like and exospheric sensillae. The mechanism of their action, apparently, is associated with displacements occurring in the cuticular apparatus with changes in temperature and/or air saturation with water vapor. The localization of the sensillae in the cuticular depressions of the exoskeleton of the antennae ensures their protection against possible mechanical damage arising, for example, at the contacts of the antennae.

Coelospheric sensillae are characterized by the generation of two types of spontaneous impulses that differ in amplitude (Lacher, 1964). High-amplitude impulses range from 0.3 to 0.4 mV, and low-amplitude – from 0.1 to 0.2 mV. When the relative humidity of air reaches 64%, the frequency of high-amplitude pulsation is about 17 Hz, and low-amplitude – 7 Hz. Under the influence of an increase in humidity from 64 to 95% in 0.2 s, the frequency of high-amplitude pulses increases to 40 Hz and that of low-amplitude pulses increases to 35 Hz.

After 1 s of the specified increase in humidity, the frequency of high- and low-amplitude pulsations decreases, respectively, to 25 and 20 Hz. In response to a decrease in humidity from 64% to 5%, the frequency of high-amplitude pulses rises to 45 Hz, gradually shrinking by the end of the first second to 30 Hz. During this time, the frequency of low-amplitude pulses decreases from 12 to 6 Hz. But when increasing the humidity from 5% to 64%, the frequency of high-amplitude pulses decreases from about 35 to 17 Hz. At the same time, low-amplitude impulses are first increased and then discharged.

It is possible to develop a conditioned reflex of bees to a certain air humidity (Kiechle, 1961). The threshold of sensitivity to changes in relative humidity is at the level of 8%.

In ethological experiments with a sequential amputation of antenna segments of the bees, it was established that hygroreceptors are located on eight distal segments. At the same time, the bees do not lose the ability to orient themselves on the humidity gradient with the complete amputation of one of the antennae, when the other has only four intact segments. However, with the amputation of each of the eight-antenna segments, the probability of detecting a bait at a reference point with air humidity known to bees (Petri dishes with a salt solution of a certain concentration) decreased (Kieche, 1961).

The probability of a correct detection of the bait by the relative humidity of the bees after the amputation of one antenna decreased from an average of 72.2 to 70.7%. When one antenna and 1-3 segments were removed from another antenna, the probability of detecting bait decreased to 53.8%. The bees with three segments on one antenna or on two antennae did not find bait according to local air humidity.

Variability of water content in the bee’s body

Exogenous water. Despite insignificant variation in the body size and other morphometric characteristics of worker honey bees (Eskov, 1995a; Eskov, Eskova, 2014), their body mass and water content may vary strongly. These depend on the fullness of the crop and rectum (Eskov, 1995a; Eskov, Eskova, 2013). The bee’s crop is essentially a container used for the transportation of carbohydrate food and water and for the primary processing of nectar and other dissolved carbohydrates into honey. The rectum serves for the prolonged storage of excrements and also for the accumulation of metabolic water under adverse conditions (Eskov, 1995a).

Exogenous water is mostly transported into the nest with trophic substrates. In particular, flower nectar brought into the hive contains up to 70–90% water; flower pollen, from 0.7 to 16.3%; and ripe honey, 18–20% (Mladenov, 1992). In addition, some water is brought into the nest by water carrier bees (Frisch, 1965; Eskov, 1983). Similarly to other insects, endogenous water in the organisms of bees is produced by the metabolism of internal energy resources that are mainly stored as lipids and glycogen (Tyshchenko, 1976; Li, Zachariassen, 2006); endogenous water comprises a relatively small fraction of the total water content.

Thus, the water content of the bee's body depends on many factors and reflects the seasonal weather conditions as well as the physiological state of the insect. The goal of this work is to analyze the previously obtained data and to estimate the effect of various factors on the water content of the bee organism and its changes at different levels of water availability.

Bees' water requirements. Unlike many other animals, bees do not drink water to satisfy their thirst. They need water for diluting carbohydrate food and for cooling their bodies in the case of overheating.

Much water is used for diluting larval food. Early instar larvae feed on royal jelly that is secreted by the hypopharyngeal glands of the worker bees and contains 60–70% water (Shkenderov, Ivanov, 1985). Late instar larvae feed on diluted honey or on nectar mixed with pollen, which they obtain during trophic contacts with adult worker bees.

The bee larvae require great amounts of low concentration food. The consumption of water by the bee colony is positively correlated with the number of larvae in it (Eskov, 1977, 1983, 1995a). Under similar ecological conditions, the daily water consumption of a bee colony decreased from 100 ± 9 to 40 ± 5 ml as the fraction of larvae decreased from 67 to 13%.

The colony requirements for additional exogenous water depend on the water content in the food. If the bees feed only on the carbohydrates stored in the nest, their water requirements increase with the concentration of the food substrate. For example, a bee colony kept in a closed cage at 20.5 ± 2 °C and supplied with a 45% sucrose solution consumed 10–20 ml of water daily. When the 45% sucrose solution was replaced with an 80% solution, daily water consumption increased to 50–80 ml (Praagh, 1975).

Water consumption is strongly affected by the ambient temperature. At temperatures of 17–28 °C and with a low productivity of the foraging area, a bee colony consumed on average 124 ± 21 ml of water per day per 1 kg of live weight. However, the daily water consumption of the same colony decreased to 60 ± 11 ml when the temperatures dropped to 2.5 ± 0.5 °C (Eskov, 1995a).

Water is delivered to the nest by water carriers, a non-specialized group of middle-aged and older worker bees that is formed and activated in cases of nest overheating and/or a shortage of diluted carbohydrate food. At the optimal ambient temperatures for the colony (24–28 °C), water carrier bees are activated when their gustatory receptors detect concentrated carbohydrates during trophic contacts with the stored food. As the crop content of the receiver bees gets diluted, they become less attracted to water carrier bees; when the foragers start bringing low-concentration carbohydrate food into the nest, the activity of water carriers ceases (Eskov, 1992).

Using their hygroreceptors (Kiechle, 1961), water carrier bees locate sources of water, fill their crops, return to the nest and, similarly to regular foragers, perform dances to recruit other bees for carrying water. The direction to the discovered water sources is encoded in the oriented dynamic postures accompanied by the lateral wagging of the abdomen. The distance to the source is encoded by the duration of the oscillating sound signal (Eskov, 2013).

Similarly to foragers, water carrier bees mostly use water sources located not far from the nest. The greatest recorded distance traveled in search of water is 2,337 m, and the mean distance from the hive to the water source is 88.9 m (Gary et al., 1979).

The behavior of water carrier bees depends on the temperature. The lowest temperature at which the bees may visit the known nearby waterer (within several meters from the nest) is 6 °C; this is often observed during prolonged cold spells in spring and autumn. When these bees leave the nest, they carry 7 mg on average in their crops, or up to 40 mg of honey as reserve food. Since under such conditions bees face a high risk of entering a chill coma, they stay at the waterer for a short time only and collect little water, due to the low rate of crop filling. At 7 ± 1 °C, the bees stay at the waterer for 10–20 s and collect 4.7 ± 1.5 mg on average, up to a maximum of 10 mg of water at a rate of 0.5 ± 0.1 mg/s (Eskov, Kostrova, 1996).

The activity of water carrier bees increases at higher temperatures: they leave the hive more frequently, stay at the waterer for longer, and collect more water. The reserve of carbohydrate food in their crops decreases from 4.8 mg (maximum 11 mg) at 12 ± 2 °C to 2.1 mg (maximum 8 mg) at 22 ± 1 °C, and at 27 ± 2 °C most bees arrive at the waterer with empty crops. An increase in temperature is also accompanied by a decrease in the carbohydrate concentration in the crops of the bees visiting water sources.

The concentration drops from 40 ± 10% at 7 ± 1 °C to 23 ± 9% at 12 ± 2 °C and 4.4 ± 1.9% at 22 ± 1 °C (Fig. 32).

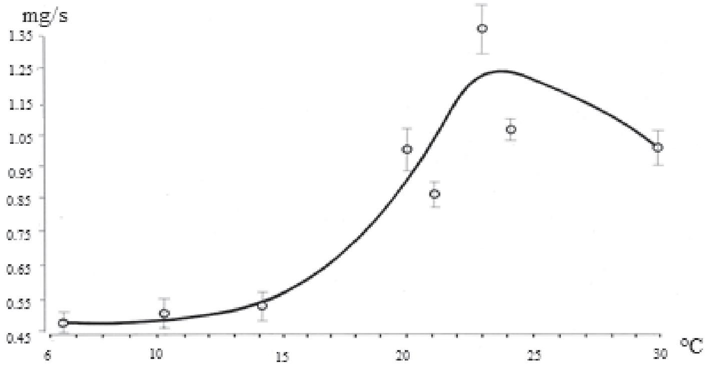

Fig. 32. The relationship between temperature (X-axis) and the rate of crop filling (Y-axis).

The crop filling rate shows a nonlinear relation to the temperature. As the temperature rises from 6 to 23 °C, the crop filling rate increases by 2.8 times. A further increase in temperature slows the process down: at 23.5 °C the crop filling rate reaches 1.37 ± 0.08 mg/s, whereas at 29–30 °C it decreases to 1.04 ± 0.03 mg/s, or by 1.32 times. Correspondingly, the amount of water delivered by the bee after a single visit to the waterer decreases from an average of 44.3 mg (maximum 67 mg) at 23 °C to 34.4 mg (maximum 47 mg) at 30 °C.

The crop is filled in several stages, the intervals between which become shorter at higher temperatures. The first interval is the longest; it is reduced on average from 16 to 5 s as the temperature grows from 13 to 23 °C, and becomes only 1–3 s long at 26–31 °C. The number of intervals also decreases from 7 at 11–17 °C to 2 at 26–31 °C. As a result, the total duration of crop filling decreases by approximately 20 times as the temperature grows from 10 to 30 °C (Eskov, 1992).

Higher ambient temperatures stimulate more frequent visits to the water source. The mean interval between visits of bees to the source of distilled water was 375 s at 10–15 °C, 241 s at 20–25 °C, and 167 s at 28–31 °C.

Water content of the digestive tract. The substrates filling the digestive tract (crop, midgut, and rectum) of the bee vary considerably in mass and water content depending on the physiological state of the insect and the external conditions. The highest variation is observed in the crop and rectum (Eskov, 1995a).

The crop. The mass of an empty crop of a honey bee is 0.6 ± 0.1 mg, whereas that of a filled crop may reach 60 mg or even slightly more. The amount of water in the crop depends on its content in the food; the crop is rarely filled with pure water. The bees feeding on carbohydrate solutions of different concentrations showed a considerable discrepancy between the water content in the food and in the crops (Fig. 33). For instance, the sucrose concentration in the crops of bees which had been feeding on 20% sucrose for 24 h after exclusion varied from 11 to 16%, and in those that had been feeding on 60% sucrose, it varied from 22 to 50%. After 3 days of consuming these solutions, the sucrose concentration in the crops was 15–32% and 36–65%, respectively. In middle-aged bees kept in cages for 2 weeks at 27 °C and fed on a 20% sucrose solution, the sucrose concentration in the crops increased on average to 28.4 ± 4.2%, whereas in bees fed on 60% sucrose it decreased to 49.1 ± 9%.

Although the properties of the substrates filling the bee crops are highly variable, the crop water content changes in a regular pattern during ontogenesis. On the completion of their postembryonic development, bees already have a certain amount of liquid in their crops. Their crops weigh 7 to 10 mg and contain 97–99% water, which is used up in the first hours of the adult stage when the young bees start consuming carbohydrate food. The crop water content by the end of the first day of the adult life of summer-generation bees is 79.6 ± 12.4%, and it decreases to 49.6 ± 19.7% in middle-aged bees.

In the annual cycle of the bee colony, the crop water content of overwintering bees reveals a relatively low variation because these bees are all in a similar physiological state. Besides, overwintering bees have a mostly uniform diet of honey that usually contains no more than 18–22% of water. However, honey gets gradually diluted in the crops: bees overwintering at 2 ± 2 °C contained on average 32 ± 6% water in their crops at the beginning of December, whereas by the middle of January the crop water content increased to 35 ± 7%. Water for dilution is obtained through reabsorption by the crop walls from the surrounding hemolymph.

The midgut. Unfed bees at the beginning of the adult stage contain 76.5 ± 8.7% water in their midguts. As a result of feeding on 20% or 60% sucrose for 3 days, the midgut water content increased to 82.8 ± 7.9% and 77 ± 7.1%, respectively. The midgut water content in middle-aged and old bees also changes: their feeding on 20 and 60% sucrose for 1 day was accompanied by a decrease in the water content to 78.6 ± 8.4 and 74.3 ±

7.6%, respectively. The midgut water content in overwintering bees feeding on honey was maintained at 74.9 ± 12.4%.

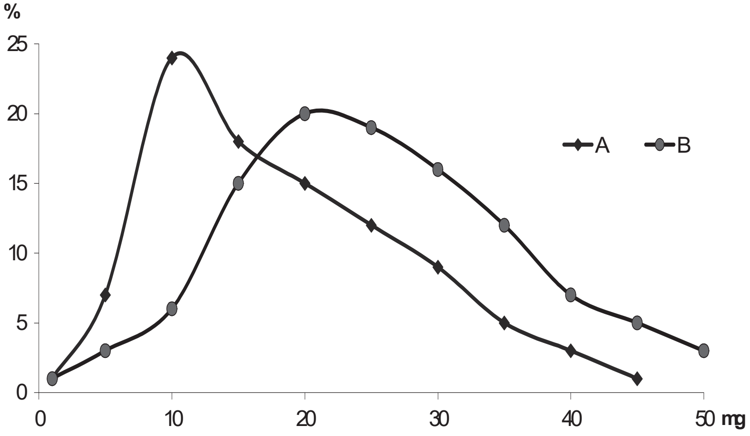

Fig. 33. Water content in crops of bees that had unrestricted access to 60% (A) or 20% (B) sucrose solutions for 3 days (on the Y-axis, % representation of bees; on the X-axis – mass of crop's content).

Similarly to worker bees, drones also have a relatively high midgut water content at the beginning of the adult stage: 84.1 ± 9.1% on average. By the time of their sexual maturation, i.e. by the age of 14 ± 1 days, this parameter decreases to 73.7 ± 13.2%, and it is then maintained approximately at the same level.

The rectum. The water content in the rectum is also subject to age-related and seasonal variation. The rectum of the bee after its emergence from the brood cell contains on average 97.5 ± 1.5% water, a value similar to the water content in the crop. After a day of feeding on a 20 or 60% sucrose solution, the rectum water content decreases to 86.9 ± 4.3% and 85.5 ± 4.2%, respectively.

The rectum water content in middle-aged and old bees varies over a wide range. For example, in cases of low productivity of the forage area, the recta of 13–21-day old bees contained 85.5 ± 8.8% water. When bees from the same colonies were placed in cages and provided with 20% sucrose or glucose for 7 days, their rectum water content increased to 92.1 ± 4.4% or 90.4 ± 5.5%, respectively; if they consumed 60% solutions of the same sugars, the corresponding values were reduced to 75.5 ± 6.6 or 79.5 ± 7.4%.

In the annual cycle, the lowest rectum water content (67.8 ± 3.4%) was recorded at the beginning of overwintering, which coincided with the interruption of the queens' reproductive function (Eskov, 1997, 2003). This parameter increases by the end of overwintering. Under favorable conditions, when most bees survive until spring, the water content increases on average by 4.2 ± 1.1%; if 30 or 80% of bees are eliminated, the water content increases by 11.3 ± 2.2 and 17.4 ± 3.4%, respectively.

In the second half of the overwintering period, the reproductive activity of the queens is usually resumed, and brood appear in the nest. In relatively small colonies, the amount of brood is inversely related to the water content in the recta: in particular, the correlation coefficient was 0.65 ± 0.14 for colonies comprising about 15 thousand adult bees. The correlation is weaker for larger colonies and virtually nonexistent for colonies with 25–30 thousand bees. The highest rectum water content in drones (94.3 ± 5.2%) is observed in freshly enclosed individuals. This parameter subsequently decreases, mostly during flights: it was 84.1 ± 4.1% in drones leaving the hive and 80.8 ± 3.8% in those returning to it.

The effects of hyperthermia. The composition and concentration of the crop content affect the rate of its consumption and the resistance of bees to hyperthermia (Eskov, 1992; Eskov, Babkina, 1990). The bees whose crops are filled with pure water are the most resistant to hyperthermia. Their resistance is reduced as the crop water content decreases. In particular, bees with crops containing 60% sucrose survived for 72 ± 18 min at 50 °C, those containing 20% sucrose survived for 98 ± 16 min, and those with water-filled crops survived for 112 ± 12 min.

The bees subjected to hyperthermia non-uniformly use nutrients stored in their bodies (mostly in the hemolymph) and crops. At the beginning of the hypothermic period, they mostly use nutrients deposited in their bodies, and later in the crops. The rate of consumption of the carbohydrate reserves from the crops is affected by their water content (Eskov, Babkina, 1990). At 50 °C, the mass of crops filled with 30% sucrose decreases at an average rate of 0.13 mg/min during the first 25 min and at 0.28 mg/min during the subsequent 50 min. Under the same conditions, the mass of crops filled with 60% sucrose decreases at 0.27 and 0.43 mg/min, respectively (Fig. 34).

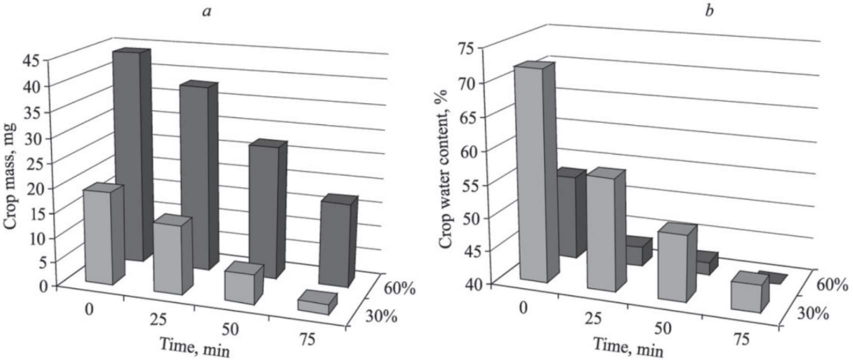

Fig. 34. Dynamics of the crop mass (a) and crop water content (b) of honey bees at 50 °C. Before hyperthermia, bees consumed a 30% or 60% sucrose solution.

Less concentrated food contains greater amounts of extractable water. The water content in the crops of bees fed on 60% sucrose decreased on average by 6.2 ± 0.3% during 75 min at 50 °C. Under the same conditions, the water content in the crops which initially contained 30% sucrose decreased by 27.8 ± 2.1%; therefore, a twofold dilution of sucrose allowed the bees to use 4.5 times as much water for cooling.

High temperatures disturb the functioning of the rectal glands, which are normally responsible for water reabsorption from the rectum (Eskov et al., 1986). For example, the rectum water content decreased by 5.4 ± 2.0% in 6 h at 45 °C, and by 0.9 ± 0.1% in 1 h at 50 °C.

Thus, a twofold decrease in the concentration of sucrose in the content of the goiter increases life expectancy under conditions of hyperthermia by 1.3 times (Eskov, 1995a).

The effects of hypoxia. Similarly to the development of mechanisms of nest temperature regulation (Eskov, 2003), the establishment of a tolerance to hypoxia has been a prerequisite for the dispersal of bees in temperate and cold climatic zones (Eskov, Eskova, 2011; Eskov et al., 2013, 2014). However, short-term hypoxia affects the age-related dynamics of the functional specialization of worker bees (Skowronek, 1976), whereas queens lose their motivation for nuptial flights and remain virgin (Mackensen, 1947).

Hypoxia affects the mass of different parts of the digestive tract differently. During anesthesia, the mass of the crop decreases while that of the rectum increases. The crop mass in bees kept in a CO_2 atmosphere decreased by 23.3, 37.9, 48.5, and 55.4% after 1, 2, 3, and 5 h, respectively; this was accompanied by a decrease in the crop water content and by the filling of the recta. Hypoxia in an N_2 atmosphere affects water loss by the bee rectum in a similar way (Table 7).

The transport of water from the crop to the rectum probably takes place during recovery from anesthesia. Correspondingly, the decrease in the crop mass and the filling of the rectum are directly related to the duration of anesthesia.

Table 7. Dynamics of mass and water content of the crop and rectum of bees subjected to hypoxia in an N_2 or CO_2 atmosphere.

Time (h)	Crop		Rectum	
	Mass (mg)	Water content (%)	Mass (mg)	Water content (%)
	M ± *m* *lim*	*M* ± *m* *lim*	*M* ± *m* *lim*	*M* ± *m* *lim*
	Initial conditions			
	10.3 ± 0.85 6.3-19	64.1 ± 1.66 23-81.6	7.6 ± 1.17 3.4-30.4	66.9 ± 1.95 40-83.3
In an atmosphere of N_2				
1	7.1 ± 0.42 4.1-14.3	61.8 ± 2.12 36.6-80.3	8.2 ± 0.77 1.7-16.6	67.3 ± 2.17 43-94.1
2	5.2 ± 0.14 4.2-12.1	54.5 ± 1.91 32.5-79.1	11.7 ± 1.49 4.1-31	67.9 ± 3.77 15-90
3	4.2 ± 0.42 2.5-10.6	53.4 ± 2.74 23.7-78.3	12.9 ± 0.78 2.7-19	68.3 ± 2.87 25.8-89.1
5	3.6 ± 0.43 2.1-10.8	50.2 ± 2.63 31.2-74.3	13.7 ± 1.18 6-2-1.2	69.5 ± 2.24 44.6-87.9
In an atmosphere of CO_2				
1	7.9 ± 0.14 2-6.6	56.4 ± 1.55 20-66.6	11.2 ± 1.46 2-28.9	67.1 ± 2.93 40-85.1
2	6.4 ± 0.26 2.3-10.7	53.6 ± 1.19 42.2-86.7	12.4 ± 1.22 5-31	68.3 ± 1.92 61.2-93.6
3	5.3 ± 0.45 2.7-13.1	52.9 ± 2.85 21.8-78.4	16.9 ± 1.32 3.7-24.6	69.7 ± 2.34 27.8-82.4
5	4.8 ± 0.47 1.9-12.4	50.1 ± 2.45 38.4-87.8	17.5 ± 1.78 2.5-34	7.9 ± 2.66 30-86.9

Endogenous water

Endogenous (metabolic) water is formed in the body of bees during the oxidation of carbohydrates, fats and proteins. One of the methods for determining the content of endogenous water in the organs and body parts of living organisms is based on their drying to a constant weight. The water content is determined by the ratio of the mass of raw and dried organs.

Age and seasonal variability. The body parts of a bee differ in structure and function. Differences in seasonal and age-related variability of water content in the head, thoracic, and abdominal regions of adults are associated with this.

Head regions. The water content of the bee's head decreases from the beginning of the adult stage to middle and old age. In particular, the heads of bees after their emergence from the brood cells contained on average 76 ± 3.3% of water, whereas by the 15th day the water content was reduced by 7.2%, and by the 30th day, by 8.8%. The water content of the consumed carbohydrate food had no detectable influence on the dynamics of this process. For instance, the water content of the heads of bees consuming 20% sucrose decreased to 73.3 ± 2.9% in 1 day and to 71.2 ± 2.7% in 3 days; in the bees consuming 60% sucrose it decreased to 73.4 ± 3.1 and 71.3 ± 2.8%, respectively.

The head's water content showed no considerable changes in the course of overwintering. In a successfully overwintering colony, this parameter increased on average from 68.4 ± 2.7 to 69.7 ± 2.9%.

The highest water content in drones is observed at the beginning of the adult stage. From the first hours after emergence to the age of sexual maturation (approximately 14 days), this parameter decreases from 77.5 ± 3.7 to 71.8 ± 2.9%, i.e. by 5.7%, after which it may only insignificantly change in either direction.

The thorax. The water content in the thorax of a worker bee depends on its age as well as on the concentration of the carbohydrate food. The thoracic water content was 75.4 ± 1.9% at the beginning of the adult stage; in bees fed on 20% sucrose, it decreased by 2.5% in 1 day, by 3.8% in 2 days, and by 4.7% in 3 days. A similar trend was observed in bees consuming 60% sucrose, in which the water content decreased by 3.1, 4 and 5%, respectively.

In bees of the spring and summer generations living in their colonies, the thoracic water content is reduced to 66.6 ± 1.7% by middle age and to 65.5 ± 1.6% by old age. This parameter increases by no more than 1% during overwintering.

The thoracic water content in drones is relatively high at the beginning of the adult stage and decreases from 77.1 ± 3.1 on the first day to 67.6 ± 2.3% by the end of the sexual maturation period.

The abdomen. Both worker bees and drones reveal an age-related decrease in abdominal water content, similar to that in the head and thorax. From the beginning of the adult stage to complete sexual maturation, the abdominal water content in drones is reduced from 79.6 ± 3.9 to 69.5 ± 2.8%, i.e. by 10.1%. Similar changes occur in worker bees of the spring and summer generations. By contrast, during overwintering, the water content changes only insignificantly (by approximately 1%).

Hypo- and hyperthermia under the conditions of hypoxia. During overwintering, the bees on the periphery of the nest are cooled down to 10–12 °C while those in the heat center of the colony are heated to 34–37 °C (Eskov, Toboev, 2009a). Overwintering bees also experience hypoxia (Eskov, 1995a).

Hypothermia. The cooling of a bee down to the state of chill coma is usually accompanied by a decrease in its body water content (Fig. 35). In a normal atmosphere, the bee's head with an initial water content of 73.7 ± 0.59% loses an average of 3.7% of water after 24 h and 8.2% after 48 h of exposure at 0 °C. The replacement of normal air with CO_2 insignificantly slows down this process, and water loss is accordingly reduced to 3.6 and 7.8%, respectively.

The thorax differs from the head in having a lower water loss under the conditions of cooling down to 0 °C: its water content decreased from an initial value of 67.7 ± 0.49% by 5.6% in 48 h. Similar to the head, this process was slightly slowed down in a CO_2 atmosphere, where water loss was 5.1% in 2 days (Eskov et al., 2013).

Hyperthermia. The head, thorax, and abdomen differ in their water loss parameters during hyperthermia. Other conditions being equal, the head loses considerably more water than the thorax and abdomen. For instance, in bees exposed to 50 °C for 60 min immediately after eclosion, the water content of the head decreased by 2.7 ± 0.3%; that of the thorax, by 0.4 ± 0.06%; and that of the abdomen, by 0.6 ± 0.08%. In 0.5-day-old bees from the same generation, the same hyperthermic exposure reduced the water content of the head by 6.8 ± 0.4%; that of the thorax, by 1.7 ± 0.09%; and that of the abdomen, by 1.8 ± 0.1%.

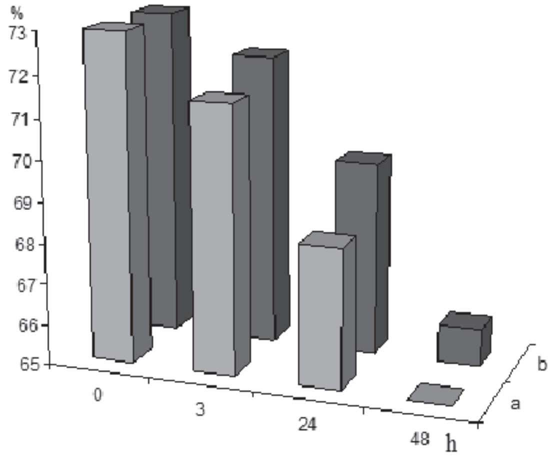

Fig. 35. Dynamics of the water content in the heads of bees in the state of chill coma at 0 °C in a natural atmosphere (a) and in a CO_2 atmosphere (b).

Hypoxia under the chill coma. Cooling down to the state of chill coma at 0 °C and anesthesia with CO_2 blocks the bee's locomotion and affects its body mass dynamics in similar though not identical ways. After 3 h of chill coma, the mass of the bee's head, thorax, and abdomen was reduced by 1.5, 1.8, and 1.3%, respectively; the mass loss after 7 h was 3.8, 2.6, and 2%; after 24 h, 6.0, 6.7, and 6.4%; and after 48 h, 16.4, 9.5, and 15.5%, respectively.

Anesthesia with CO_2 reduced the effect of cooling on the body mass dynamics in chilled bees. In anesthetized bees, exposure to 0 °C for 3 h reduced the mass of the head, thorax, and abdomen by 0.8, 1, and 0.3%, respectively; the corresponding mass loss after 7 h was 2.3, 2.1, and 0.7%; after 24 h, 5.3, 4.1, and 2.4%; and after 48 h, 15.2, 8.2, and 11.5%, respectively. The initial mean values were 13.2 ± 0.22 mg for the head, 38.8 ± 0.54 mg for the thorax, and 68.6 ± 0.59 mg for the abdomen.

Conclusion

The development of a wide range of honey bees is associated with the acquisition of adaptations to shelter conditions in moderate and cold climates, which was largely helped by the acquisition of the adaptation of a high tolerance to hypoxia. During the period of the intensive reproduction of worker bees and drones with fluctuations in the external temperature from 5 to 35 °C, the intra-nesting temperature usually does not exceed 32.5–36.3 °C. During the warm season, the CO_2 concentration in the central zone of the nest occupied by brood varies from 0.05 to 0.15%, and during long spring-summer cooling periods it can reach 2.5–3%. In the fall, in the zones of localization of the brood completing development, the concentration of CO_2 can reach 4–6% (Eskov, 1995a).

A bee brood can normally develop only in a narrow temperature range, with optimum values of 33–34.5 °C. Deviations beyond the limits of this range by 3 and 5 °C determine the average and absolute lethal efficiency, respectively. Conversely, an excess in CO_2 concentration by two orders of magnitude relative to the CO_2 content in the hive at an optimal temperature for the bee colony has a lethal efficiency that is only slightly greater than its minimum (10%) level.

Temperature deviations from the optimal values and an increase in the CO_2 concentrations of up to 10–15% cause a significant decrease in the viability of bees, a shortening of the crops, and the partial or complete underdevelopment of wings. The shortening of wings is accompanied by a change in their symmetry because the right wing becomes longer than the left. Similarly, high concentrations of CO_2 affect changes in morphometric traits, body color, and behavior modifications of the migratory locust (Woldring et al., 1978). This reflects the convergent similarity of the physiological effects of CO_2 in remote taxonomic groups of insects.

In contrast to morphometric signs, the body weight of bees living to the adult stage, both under increasing CO_2 concentration and with decreasing temperature (Eskov, 1995a), increases. These bees are also distinguished by high water content in the head, thoracic and abdominal parts of the body, which indicates a violation of their water metabolism.

Hypoxia has the opposite effect on adult bees. In anesthesia in the atmosphere of CO_2, there is a sharp decrease in the body mass of the bees, which obviously is mainly due to water loss. These processes are intensified under the influence of an increase in temperature in the range that ensures a high level of activity of bees. But in a state of cold numbness, anesthesia slows down the dynamics of reducing the mass and water content in different parts of the body of bees. Since the respiratory function is suspended in a state of chill coma and anesthesia, the transport of water occurs mainly through the integuments of the body.

Bee colonies suffer oxygen starvation, but hypoxia helps reduce energy costs during periods unfavorable for their active life. The increased CO_2 content during wintering contributes to energy savings and, accordingly, a decrease in feed intake due to some inhibition of the metabolism (Eskov, 1995a), which is similar to a decrease in the overall metabolism and body temperature in mammals under the influence of hypoxia (Blatteis, Lutherer, 1976).

Mammals and bees react differently to the lack of oxygen in the environment, striving, essentially, to achieve an adequate goal. In mammals living in high mountains, exogenous hypoxia is compensated for by activating pulmonary ventilation, which is achieved by increasing the frequency of respiratory cycles (Arav, Smolichev, 1947; Vasiliev et al., 1974). The bees react to an increase in CO_2 content in the nest by reducing the density of aggregation and increasing the air exchange between the nesting space and the external environment by flapping their wings (Eskov, 1995a).

The effect of hypoxia on embryonic development in mammals is limited by the protective mechanisms of the maternal organism. Therefore, altitude hypoxia has little effect on the rate of development of the embryos of mammals (Metcalfe et al., 1962). Anomalies in the pregnancy of mammals under the influence of altitude hypoxia are not observed, although mortality among nascent offspring increases (Moore, Price, 1948; Weihe, 1964). Embryonic development in birds, deprived of the direct protection of maternal organisms to a greater extent than mammals, is subject to high altitude hypoxia. It, like hypothermia, inhibits metabolic activity in bird embryos. The damaging effect of high altitude hypoxia on avian embryos is expressed in the slowing of their growth and increasing mortality (Taylor, Kreutziger, 1965; Wangensteen et al., 1974). In this respect, the effect of hypoxia on the development of bees is closer to birds than to mammals.

In the class of many species of singly living insects, protection against hypoxia, as well as many other adverse effects of the environment, is provided by means of the diapause. In this state, insects live at the expense of internal reserves, being in a stationary state, which is outwardly similar to the chill coma and anesthesia under the influence of CO_2 in a honey bee. But a fixed insect represents easy prey for predators. The large progression of the reproduction of diapausing insects ensures that their populations and species survive. In contrast, the honey bee colony is weak and does not always reproduce during the annual cycle of life, which occurs in the process of sociotomy (Eskov, 1992; Eskov, Toboev, 2011a, 2011b).

Sociotomy, ensuring the resettlement of bee colonies, poses a threat to their existence, which depends on the ecological situation at the new relocation site and, especially, on the productivity of the forage area. And for resettled and maternal colonies, the most dangerous period of life is associated with wintering. At this time, the probability of survival for bee colonies depends on the availability of food, which, like the bees themselves, needs protection from predators and robbers. To do this, the bees, or at least some of them, must be in an active state, which in turn is impossible without maintaining a relatively high temperature.

The regulation of the temperature in the bees' nest is associated with the processes of thermogenesis and thermal insulation, which are achieved by aggregating the bees around the heated zone. As a result of bee compaction, a reduction in heat losses is achieved, but so is an increase in the CO_2 content in the nesting space occupied. Therefore, in the process of adapting the honey bee to temperate and cold climates, natural selection favored the development of an effective mechanism of thermoregulation and high tolerance to hypoxia.

The need of the bee colony for water and/or liquid carbohydrate feed changes noticeably in the annual cycle of life. In hot weather, water is required to cool the nest and the bees and brood in it. The cooling of a bee's body and the nesting space surrounding it is achieved by the evaporation of water by the respiratory system and the integuments of the body. The involvement of the integument in this process is confirmed by experiments, where bees that were in a state of anesthesia, under the action of hypoxia or in a state of cold numbness lost a large amount of water as a result.

The bee colony experiences an acute need for water when there are hundreds of carbohydrate forage reserves in the cells, which are represented mostly by undiluted honey. This is observed after the completion of wintering or prolonged bad weather when bees cannot fly out of the nest. In this case, water is needed mainly by the larvae that feed on liquid food. The shortage of water can be aggravated because, with the possibility of choice, forager bees prefer sources of food with highly concentrated solutions of carbohydrates, thus saving energy for the processing of these solutions into honey.

The water is delivered to the nest by a non-specialized group of aquatic bees, represented mainly by older individuals. After returning to the nest, water bees transfer it to other bees or keep it in their crops, finding themselves in areas exposed to intense overheating. A rapid satisfaction of the colony's need for water is achieved by the fact that water bees use a mobile alarm system. This includes targeted movements and sound signals, the duration of which correlates with the distance to the detected water source. Often, after visiting a water source and returning to the nest, the bees mobilized by water carriers inform other bees about its location. This achieves an increase in the number of aquatic bees as long as the colony needs water. After satisfying their need for it, the attitude of attraction towards the water bees changes to an indifferent one and they switch to performing other works.

During the period of preparation for wintering, the content of endogenous water in the body of the bees decreases. This increases the resistance of bees to freezing, which is characteristic of many wintering insects (Ushatinskaya, 1957). The development of the honey bee zones of temperate and cold climates is associated, in particular, with the development of ethological mechanisms of regulation of the intra-nesting temperature and the accumulation of reserves of food in the nest, which provide for a long wintering period. The reproductive diapause of the queens contributes to the economical use of energy resources during wintering, during which the bee colony stops the reproduction of brood, which requires the additional consumption of feed, water and energy costs of working bees to heat the nest (Eskov, 1997).

The lack of water at the beginning of wintering suppresses hormonal and reproductive functions in females. During the wintering period, the water formed during the consumption of the feed, when resorbing into the crops, dilutes the honey contained in them, which is similar to the consumption of low-concentration carbohydrate feed. This stimulates the resumption of the functioning of the hypopharyngeal glands secreting royal jelly, necessary for ovulating queens and young larvae, in worker bees. From this time, usually in the middle or second half of wintering, the queen begins egg ovipositioning and brood breeds appear in the nests of bee colonies.

Adult bees can consume food that varies in carbohydrate concentration by about an order of magnitude. Moreover, the water content in the feed significantly affects the duration of their life. Large groups of bees kept in cages that are similar in age and physiological condition, when consuming 60% sucrose, live 2.5–3 times longer than those that consume 20% sucrose. This confirms the effect of the concentration of carbohydrate feed consumed by bees on their lifespan.

CHAPTER FOUR

MOBILE COMMUNICATION SIGNALS

The coordination of purposeful flights of bees is provided by mobile communication signals. They are used in the system of the trophic support of bee colonies, the delivery of water, and the detection of new places for settlement. The mobility of the nesting behavior of bees ensures their adaptation to daily and seasonal changes in the ecological situation.

Locomotor activity of bees

Intra-nesting behavior. In bees that are within the nesting space, locomotor activity varies depending on the physiological state and the ecological situation that influence the adequate response of bees to external and internal stimuli.

The intra-nesting behavior of worker bees includes feeding and heating the brood, building and reconstructing the damaged honeycombs, processing nectar into honey and filling the fodder cells with it, which along with the state of the environment depends on the locomotor activity of adults.

In the spring-summer period, the daily activity of the bees is largely associated with changes in temperature, light and the productivity of the feeding area. The spectral structure and the intensity of sounds generated by bees depend on the activity of bees.

In the spectrum of sounds generated by the bee colony, there are two ranges of intense components. The first of them is generated by the flapping of the wings of bees who are involved in cooling the nest. With a low activity of ventilating bees, the peak of the intensity of this range is in the region of 75–85 Hz. Under the influence of an increase in the internal nesting temperature to 30–33 °C, the intensive components in the sounds of the bee ventilators shift by 110–120 Hz, and at 40 °C they reach 145–155 Hz.

The second range of intensive components is associated with the activity of bees engaged in the delivery of food and the heating of the brood. With daily temperature fluctuations in the range of 16–26 °C and a low feed area productivity (delivery to the hive of 0.5 ± 0.2 kg of feed) during the daytime hours, the maximum spectral energy of the sounds of the bee colony is in the range of 230–255 Hz. By the end of the day, as the illumination decreases to 1–3 lx, the energy maximum in the sound spectrum will be mixed at 220–240 Hz and its intensity will decrease by 4–5 dB. In the morning hours, with an increase in illumination to 10–25 lx, the energy maximum of the sound spectrum shifts to the high-frequency region by 15–25 Hz, and its intensity increases by 1.5–2.5 dB. Under the influence of increasing the productivity of the feed area by 2–3 times, activating forager bees, the intensive components of the second range are shifted to the high-frequency region by 14–23 Hz, and their intensity increases by 4–6 dB.

The preparation of colonies for sociotomy is accompanied by the appearance of intensive components in the range of 210–230 Hz. Their intensity increases with the approaching day of swarming. 3–5 days before the swarm exits, the components of this range are 6–9 dB higher than the adjacent high-frequency region of the spectrum.

Changes in the intra-nest microclimate coincide with diurnal fluctuations in illumination, which are most likely before sunrise on days that are favorable for flight. An increase in illumination is usually accompanied by an increase in temperature and CO_2 concentration. Thus, at 16–18 °C and with illumination reaching 20–50 lx, the temperature at the brood increases by 0.7–0.8 °C, and the CO_2 concentration in this and the central zones of the nest – by 0.1–0.25%. In contrast, during periods of cooling at 7 ± 0.4 °C, the marked increase in light intensity effects only an average increase in CO_2 concentration of 0.27%. A further increase in illumination at low temperatures, preventing flight, increases the variability of CO_2 concentration.

In the autumn-winter period, the bees wintering in the open air or indoors did not demonstrate a change in the structure of the sounds they generated due to diurnal changes in illumination. Often, the activation of bees can be stimulated by a lack of food, disease progression, an increase in external temperature, etc. The amplification of sounds during the activation of bees is usually accompanied by a shift of the energy maximum to the high-frequency region (Eskov, 1979).

Bees wintering in the open air, which corresponds to the natural conditions of their life, do not react to an increase in illumination, which on frosty sunny days reaches 8,000-100,000 lux. But they are activated and can fly out of the hive during periods of thaw when the temperature rises to 7–14 °C, with illumination not exceeding 1,000–2,000 lux. The relatively low temperature provokes sorties of Caucasian honey bees.

European dark bees require higher temperatures for flights. These differences are related to the number of bees in colonies and adaptation to typical habitat conditions. Large colonies of European dark bees are biologically inappropriate to respond to minor short-term temperature rises, since the activation of hibernating bees is associated with high energy costs. Relatively small colonies of Caucasian bees react to minor thaws. This will allow the bees to make cleaning flights, and, in the second half of wintering, to replenish fodder stocks in the presence of flowering honey plants.

Summer activity. Bee flights out from the hive are limited mainly by light and temperature. At a time that is favorable for replenishing feed stocks, some bees are activated long before dawn with illumination that reaches only 0.05 lux. These bees migrate to the entrance, but do not fly out of the hive. The minimum illumination at which first departures are possible depends on the distance to the sources of food known to bees (Schricker, 1965). Bees with flavored sucrose solutions, located 25–50 m from the hive, can be visited by bees at an illumination of 0.1–0.2 lux. A visit to the feeders at 1,000 m from the hive is possible with an illumination of 3 lux, and at 3,875 m – not less than 15 lux. The aromatization of food acts as an additional guideline in the process of the detection of bee feeders by bees.

The level of illumination at which bees stop flying in the evening before the onset of darkness depends on a number of factors. Among them is the distance from the hive to the source of food. With an increase in this distance, the bees complete sorties at higher levels of illumination. With a favorable meteorological situation, the bees that had been feeding for several days with a two-molar sucrose solution at a distance of 50 m from the hive completed departures when the illumination decreased to 4 lux. The bees of the same colony that visited the feeders with the same sugar solution that were 1,000 m from the hive completed departures at 16 lux, and at 3,875 m – at 216 lux (Schricker, 1965).

Equally important is the quality of the feed. Bees stopped flying to feeding troughs with a two-molar sucrose solution, located at a distance of 50 m from the hive, when the illumination decreased to 3.9–4.0 lux. Bees stopped flying to bee feeders with a unimolar sucrose solution located at the same distance from the hive when the illumination decreased to 7.3 lux, and to feeders with a 0.5-molar solution when it decreased to 9.4 lux (Schricker, 1965).

In the daily cycle of changes in the flight activity of bees, the increase in the morning phase and the evening decrease are clearly distinguished. The intermediate phase, which occupies the largest part of the daylight hours, is usually characterized by equality between the number of bees departing from the dwelling and the bees returning to it.

The phases of morning activation and evening inhibition of flight activity are directly dependent on the diurnal change in illumination. However, its identical levels, under conditions that do not prevent the bees from departing from the hives, can have different effects on the daily dynamics of their flight activity in different periods of the summer season.

Despite the fact that the number of bees in the hives increases from the beginning to the end of the summer, their flight activity has a pronounced downward trend. In particular, in early summer the bees began to fly out of the hives with illumination from 3 to 18 lux. In the morning, the relationship between the increase in flight activity and illumination was traced until it rose to $1 \cdot 10^4 \pm 2 \cdot 10^3$ lux. From this level of illumination, a dynamic balance was usually established between the number of bees departing and returning to the hive (Fig. 36A). At the end of the day, with an illumination of $3 \cdot 10^4 \pm 1.5 \cdot 10^3$ lux, this balance was disturbed by reducing the number of bees flying out of the hive. Departures stopped when the illumination decreased to 14 ± 6 lux (Fig. 36B).

At the end of the summer, the bees began to fly out of the hives when the illumination reached 35 ± 5 lux. An equality between the number of departing and arriving bees was established with a light level of $1 \cdot 10^4 \pm 2.4 \cdot 10^3$ lux (see Fig. 36C). The tendency for the number of arriving bees to exceed those departing was traced at the end of the day, when the illumination decreased to $6.2 \cdot 10^4 \pm 3 \cdot 0.9 \cdot 10^3$ lux, and stopped at 19 ± 6 lux (see Fig. 36D).

The decrease in the bees' use of daylight hours for flights from the beginning to the end of summer is mainly due to a change in the physiological state of bee colonies. With other things being equal, a decrease in the reproductive activity of the queen is essential, which corresponds to a decrease in the number of developing bees and their ratio to adults. The number of developing bees at the larval stage is largely associated with the need for food and water (Eskov, 1995a). Therefore, artificially reducing the number of honeycombs occupied by larvae (removal from the nest), or increasing it (introduction from other colonies), respectively reduces or increases the activity of bees that are engaged in the delivery of pollen (Free, 1967). Another stimulating factor is the presence in the nest of a large number of empty honeycomb cells. Their artificial increase or decrease, like that of brood, affects the change in flight activity of bees (Rinderer, Baxter, 1979). However, the influence of the amount of brood and empty cells is significantly lower compared with the influence of the thermal factor, the productivity of the feed area and the wind speed.

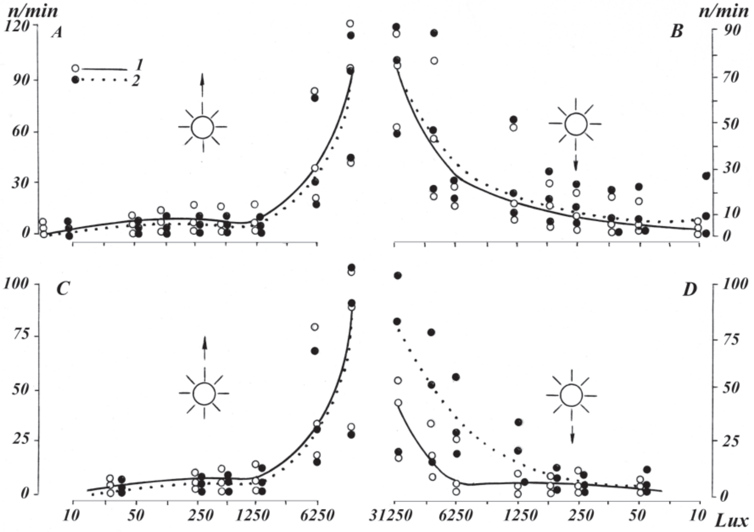

Fig. 36. The dependence of the flight activity of bees (n/min) on illumination (lx) at the end of spring-early summer (A, B) and at the end of summer (C, D): 1 – the number of bees departing from the hive; 2 – the number returning to it (A, B – morning, C, D – evening).

The daily duration of daylight hours loses influence on the dynamics of flight activity at a temperature unfavorable to flight. Regardless of the motivation of departures, they are suspended during periods of spring-summer cooling. The complete cessation of departures stimulates a decrease in temperature to 5 °C. At this temperature, on sunny days bees migrate in large numbers from the nest to the letting hole. Individual bees go out and take off, but immediately return to the hive. Those bees that remain outside the dwelling for a few minutes are subjected to a chill coma.

With high productivity of the feed area and favorable weather conditions, the bees start flights early and end them late. Their initial and final phases strictly correspond to the increase and decrease in illumination. A decrease in the productivity of the feeding area decreases the duration of the flight. They begin and end at higher light levels. If intense nectar release occurs at the beginning or end of the light in the day, then, accordingly, the phases of maximum flight activity shift.

Aircraft. The beginning of the insect development of the air environment probably occurred in the Devonian or Carboniferous period. It is assumed that wings developed as lateral processes of the thoracic tergites. Winged insects probably originated from their ancestors that lived on trees. The primordial, immobile wings made it possible for the insects to descend from trees by smoothly gliding or supporting them in the air during leaps from branch to branch (Rasnitsyn, 1980b). The improvement of the flight apparatus and the emergence of flapping wings were conducive to the active dispersal of insects, the colonization of various habitats, and their differentiation into hundreds of thousands of species. The advanced flight apparatus of modern insects allows them to migrate over considerable distances, providing for population density regulation and the mastering of new living conditions (Lorch et al., 2005; Holland et al., 2006).

The aircraft of the bee is represented by two pairs of wings, the raising and lowering of which is ensured by the deformation of the muscles of the indirect action of the mesonotum. In the phase of contraction of the transverse muscles, the back is compressed in the dorsoventral direction, and the wings are raised. Their lowering occurs during the contraction of the longitudinal muscles that raise the back. At the same time, under the action of the sclerites, the wings move along a complex trajectory around their longitudinal axes.

The wing of a flying bee (its tip) describes a sinusoidal curve of irregular shape along the flight line. In relation to the body, the tip of the wing moves on a figure-of-eight trajectory. By changing its inclination, changing the plane of the flap of wings, the flight from forward or backward movement to soaring is regulated.

Worker bees, queens, and drones of the western honey bee differ in wing size and body weight. For average European worker bees, queens and drones developing in optimal conditions, the body weight averages 112 ± 3.3 mg, 212 ± 4.1 and 251 ± 5.4 mg, and the length of the front wings – 9.37 ± 0.016, 9.37 ± 0.016 and 12.41 ± 0.031 mm respectively. But a direct dependence between these parameters is retained only at the onset of the adult stage. High values of the correlation coefficient between forewing length and body weight in worker bees, queens, and drones (0.843 ± 0.087) are observed until the bees start feeding and leaving the nest. However, although the differences in wing size are considerable, 8.1% between queens and worker bees and 24.3% between queens and drones, the differences in weight are considerably greater – by a factor of 1.87 between worker bees and queens and 2.16 between worker bees and drones.

In worker bees, drones, and, to a smaller extent, queens, the body weight can change considerably depending on their physiological state and ecological conditions. In worker bees, this parameter can change by a factor of about 1.5 as a result of the filling of the honey sac, midgut, and rectum. For the same reason, the body weight of drones decreases between leaving the nest and returning to the nest on average by 28 ± 3.4 mg, i.e. by 11% of the average body weight of drones that developed in honeycomb cells of the drone type.

Queens take flights during the breeding period in the first 7–30 days of the adult stage. Queens at this age have the lowest body weight. Ovulating queens fly without resting for relatively short distances. The physiological preparation of ovulating queens for flights includes a decrease in body size by several percent, resulting from a partial degeneration of the ovaries.

Differences in body weight and wing size between worker bees, queens, and drones lead to differences in their flying capacity, although their flying apparatuses are morphologically similar (Snodgrass, 1956). Among the castes of the western honey bee, the load on the flying apparatus during flight is especially high in worker bees. The viability of the entire bee colony depends on the flying activity of worker bees, which forage and deliver carbohydrate and protein food to the nest. This is why worker bees, with their relatively low but highly variable body weight, have acquired relatively large wings in the course of species phylogeny, which was accompanied by a functional and morphophysiological differentiation between members of bee colonies.

The results of mathematical and computer modeling of the honey bee's flight at a minimum drag and maximum wing stroke frequency suggest that the lift created by the wings should be insufficient for overcoming gravity. For instance, in a bee weighing 100 mg (with a minimum filling of the digestive tract) flying at an angle of 45° to the vector of gravity with a speed of 5 m/s, the calculated lift is only sufficient to support 88% of the body weight. Under the same conditions, if the filling of the honey sac and/or rectum is maximal and the body weight increases to 150 mg, the calculated lift supports only 70% of the body weight (Dudley, Ellington, 1990; Usherwood, Ellington, 2002).

The actual lift is probably considerably higher than the above-calculated values because of the turbulence created by the wings, which perform retrogressive translational movements in the course of flight (Usherwood, Ellington, 2002; Du, Sum, 2008). This process is also influenced by the supination and pronation of the wing planes, in which axillary sclerites participate (Snodgrass, 1956). In addition, judging by the frequency and amplitude structure of the acoustic spectra of sounds produced by flying honey bees (Eskov, 1969, 1979), their wings are subject to vibrations. The intense components of these vibrations fall within the ranges of 220–280 and 400–600 Hz. The vibrations may increase lift due to turbulence at the surface of the wings, which are covered by numerous microvilli.

Generation of sound and electric signals

In the mobile communication system, bees use sound signals transmitted through the air and/or through the reference substrate. The generation of sound signals and locomotion is often accompanied by low-frequency electric fields (EF). Tactile contacts and trofollaksis occupy a large place in communications and in maintaining the unity of the bee colony.

A broad interest in studies of signaling in honey bee communication was aroused in the mid-twentieth century by the works of an Austrian zoologist, Karl von Frisch. He proved that bees, in the process of stereotyped motility (dancing), including pendular movements of the abdomen in the lateral plane, relay information on the coordinates of the discovered source of food. This has been confirmed in numerous investigations performed by different ethologists.

Observing the dancing bees in open combs, one can – with a high degree of reliability – get a notion of the position of the feeding site visited by these bees. However, within the bee hive, it is dark, which excludes a visual perception by the bees of the locomotory postures of the dancers. This prompted the necessity of revealing other signals attending the locomotory postures. Research performed in the 1970–80s established that, in the process of dancing, the bees generate pulsing sounds. Their informative significance was doubted inasmuch as bees were not known to have an organ perceiving the acoustic vibrations of air. Apparently, the American physiologist H. Esch was quite close to revealing bees' phonoreceptors, according to the report of the XXI Congress on Beekeeping. Yet, the phonoreceptor of the honey bee was later revealed by the author of this review, after which interest among American and West European researchers in studying bee bioacoustics dropped sharply. New works were mainly concerned with the study of noise immunity and the reliability of communication. Separate investigations executed out of connection with the available data in the literature were often marked by pronounced compilativity and interpretations that were inconsistent with the results obtained.

Sound generation. The interest in studying the mechanisms of sound generation by bees was initially connected mainly with the so-called "queen's songs." Beginning in the nineteenth century, several hypotheses were developed. One of them suggested that queens generate sounds by rubbing their mandibles (Kalinsky, 1890), wings (Armbruster, 1922) or axillary plates in their bases (Snodgrass, 1925). These suggestions were refuted by the fact that queens, upon the amputation of their mandibles, wings (Eskov, 1979; Woods, 1963) and wing sclerites (Eskov, 1963), could still generate sounds.

The hypothesis of sound generation with the aid of the respiratory system has been diversely elaborated. It has been supposed that sound is generated upon air passing through stigmata as a result of the vibration of the elastic membranes thereon. The different frequencies of sounds were explained by the varying sizes of stigmata and abdominal segments (Gooding, 1951) acting as resonators. According to another suggestion, the spiracles opening and closing interrupt the air flow, and sound is generated by the principle of an air siren.

The use of the respiratory system in sound generation has not been confirmed. It has been established that air pressure in the respiratory system is insufficient for sound generation (Pringle, 1956). In addition, the blockage of the abdominal spiracles in the queen does not deprive it of the ability to generate sounds (Simpson, 1963), and the replacement of air nitrogen with helium does not change their frequency.

It has been experimentally proven that sound generation by bees is based on the use of the flight apparatus, of which the most important elements are the hemispheric scutum of the mesothorax and the elastically adjoining scutellum (Eskov, 1969). This joint deforms under the action of longitudinal and transverse indirect flight muscles. Each flight cycle of wing motion is provided by two periods of thoracic vibration with the longitudinal muscles and one period with the transverse muscles; this is confirmed by the generation of one electric pulse per 7.1 ± 0.48 contractions of the longitudinal muscles and 4.5 ± 0.19 of the transverse muscles.

The coincidence of the frequencies of thoracic vibrations and wingbeats of a flying bee determines the presence in the acoustic spectrum of two intense components, one of about 250 and the other of about 500 Hz. Wings act as resonators amplifying the transmitted thoracic vibrations. This explains the redistribution of intensity from low- to high-frequency components upon a partial or complete amputation of the wings (Fig. 37).

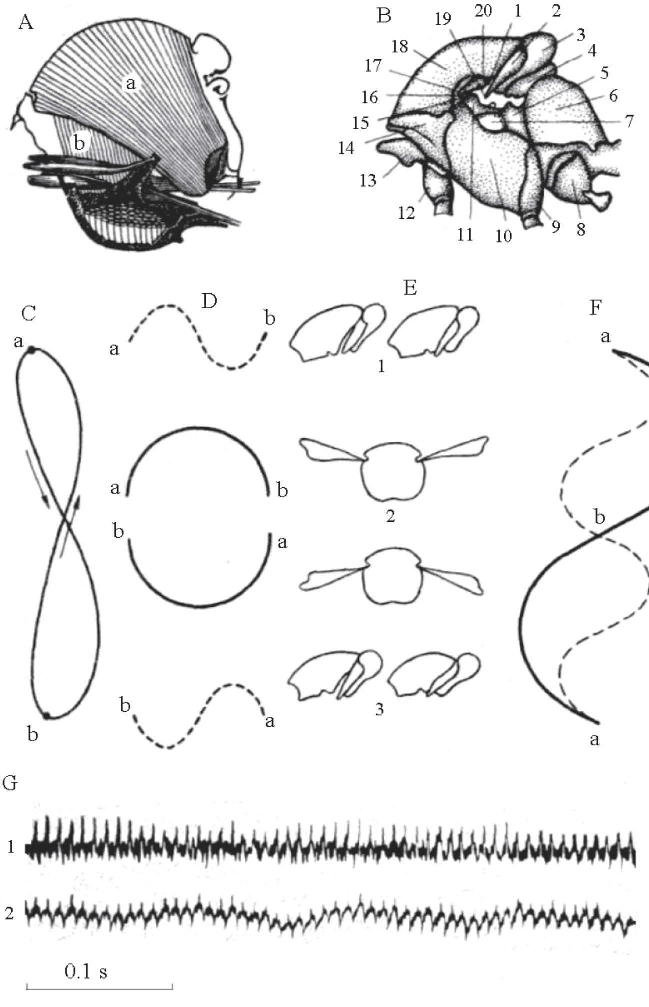

Fig. 37. The vibrations of the exoskeleton of the bee's thorax and the generation of sounds: A – deformation of the thorax by the muscles of indirect action: a – longitudinal, b – dorsoventral (transverse) muscles; B – the exoskeleton of the thorax (1–20); C – the trajectory of the movement of the wing tip of a flying bee; D, E, F – phases of deformation of the scutum and scutellum during the movement of wings (a – b, b – a); G – conjugacy of the synchronicity of the sound vibrations recorded by the microphone (1) and the vibrations of the thorax (2) recorded by the vibration sensor (Eskov, 1979).

The multifunctionality of the flight apparatus is manifested in its use for the generation of pulsed sounds that are not connected with the beating of the wings, which barely vibrate in the lateral plane. Wing amputation does not essentially affect the spectral structure of pulsed sounds. They are generated by thoracic vibrations with longitudinal muscles. This is indicated by the coincidence or precedence of their action potentials versus thoracic vibrations. Inasmuch as longitudinal muscles have a relatively high contraction frequency, they provide a generation of sounds with a maximal intensity in the range of 400–700 Hz.

The frequency of sounds generated by intact bees, with a change in intensity limited to 40 dB, may reach 8–12 kHz. The most intense spectral components of acoustic processes attending various vital processes fit into the range of 70–700 Hz, usually appearing as one narrow band, or more rarely two or three.

EF generation. Bee body covers electrify and retain an electrostatic charge. Other conditions being equal, the magnitude of the charge depends on bee activity. At 70–80% inhive air humidity, the charge of low-motile bees on the combs varies from –1.8 to +2.9 pC. Very active dancing bees carry a positive charge averaging 45.0 ± 4.3 pC and maximally 80 pC.

The magnitude of the charge of active bees is influenced by the properties of the substrate and air humidity. At 70% humidity, for bees having moved 5 cm over a surface of tin, the body charge reached 1.6 ± 0.1 pC. Upon moving the same distance on silk fabric, it was 2.9 ± 0.4 pC, on glass – 5.6 ± 0.9, on paper – 7.8 ± 0.8, on wool fabric – 11.7 ± 1.3, and on wax – 11.4 ± 1.8 pC. Raising the humidity to 90% entails a drop in bee charge by an order of magnitude. Therewith no influence is found regarding the distance flown by the bees from the food source to the hive: in those that flew 5 m, the charge was +0.98 ± 0.13 pC, the same as in those that flew 200 m (+0.96 ± 0.13 pC).

Vibrations of charged body parts create changes in the electrostatic field intensity in the space around it (Fig. 38). The shape of the electric oscillations registered by a probe a few millimeters away from the bee depends on its position relative to the moving insect or parts of its body. Upon a single change in distance over a period of reciprocating motion of the bee’s body, wings or abdomen relative to the probe, one period of oscillation of EF intensity is recorded. The oscillation frequency is doubled when, within the period of reciprocating motion of the bee’s body, the distance to the probe changes twice.

The oscillations of the parts of the charged body of a bee give rise to changes in the electrostatic field’s strength in the surrounding space (Fig. 38). The form of the electrical oscillations recorded by a probe located at a distance of several millimeters from a bee depends on its position relative to the moving insect or parts of its body. With a single change in the distance during the period of the return of the translational movement of the bee, its wings or abdomen relative to the antenna of the probe, one period of oscillation of the intensity of the EF is recorded. A doubling of the frequency of its oscillations occurs when the distance from it to the probe changes twice in one period of the reciprocating movement of a bee's body.

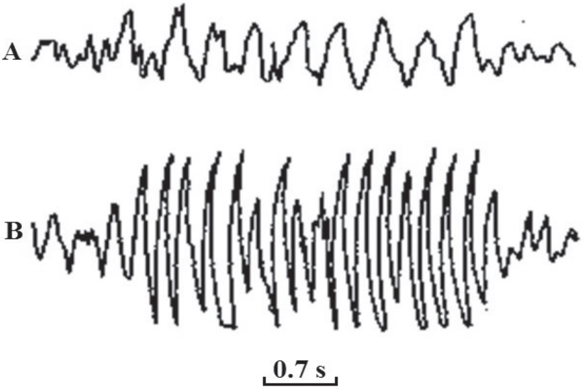

Fig. 38. The structure of the oscillations of the intensity of the electrostatic field generated by a bee-dancer (A – probe in the lateral part of the abdomen; B – probe above the belly).

Inasmuch as sound generation is coupled with wing motion, this process is usually accompanied by electric oscillations (Fig. 39). Their amplitude depends on bee charge and wing beat amplitude. At 1 cm from a bee beating its wings to air the nest, EF intensity is 0.52 ± 0.10 V/cm, while over a bee generating pulsed sounds, it is 0.19 ± 0.05 V/cm. With an increase in charge on the bee generating pulsed sounds from 15 to 100 pC, the EF oscillation amplitude grows by 2.3 times. The amplitude spectra of the electric and acoustic oscillations generated by the bee coincide.

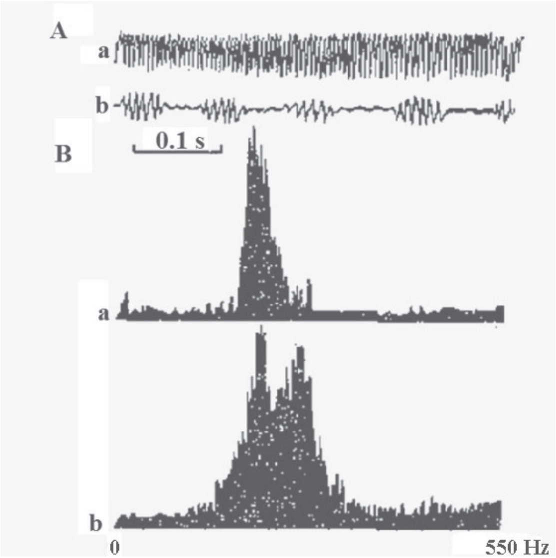

Fig. 39. The time (A) and frequency-amplitude structure (B) of electrical oscillations generated by bees flapping their wings (a) or making small movements in the lateral plane (b).

Perception of sound and electric signals

A perception of acoustic and electric oscillations by the honey bee is provided by specialized and/or universal mechanoreceptors. Acoustic signals can be transmitted and received via two channels – air and substrate. The universality of phonoreceptors manifests itself as their participation in EF perception.

Phonoreceptors. Low-frequency acoustic signals propagating in the air are perceived by fast-adapting trichoid sensillae (Fig. 40). They are positioned laterally on the bee’s head between the compound eyes and the occipital suture. The role of primary transducers of air acoustic oscillations is played by the hairs elastically mounted in the cuticular capsules. The hairs of a worker bee are 500–900 µm long (Eskov, 1975).

The hair base in the capsule is 9.20 ± 0.43 µm in diameter, suspended by means of the articular membrane. The outer side of the latter is adjacent to the capsule walls (inner diameter 24.10 ± 1.07 µm). The external protrusion of the cuticle gives rise to a vallum that delimits hair deflection (Fig. 40).

Each hair is innervated by one bipolar neuron residing under the capsule. The neuron is 23.0 ± 1.8 μm long and 9.0 ± 0.7 μm wide. A dendrite of the receptor cell enters the capsule through the hole in the inner (proximal) vallum and attaches to the hair, penetrating its base through the channel. The axon of the sensor cell, circumventing the inner vallum, passes along the cuticular wall.

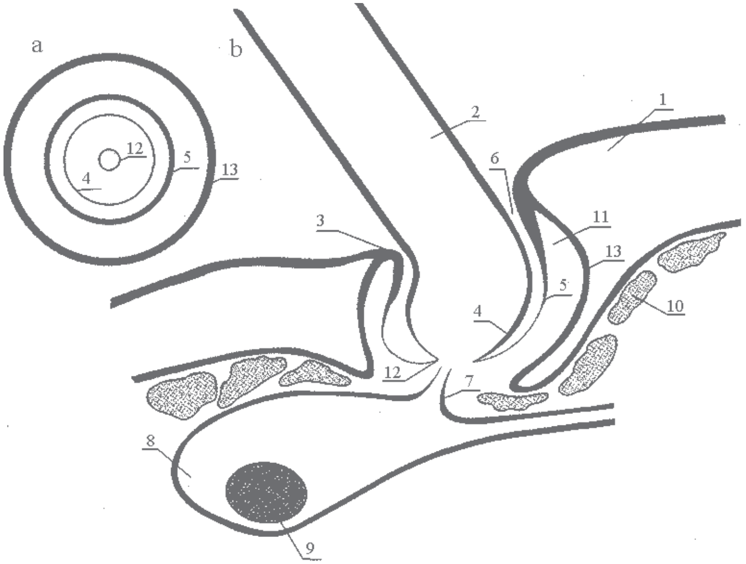

Fig. 40. Schematic representation of a fast-adapting trichoid sensilla of the honey bee: (a) transverse section through the central part of the cuticular capsule; (b) longitudinal section (1 – cuticle, 2 – hair proximal part, 3 – neck, 4 – base, 5 – articular membrane, 6 – entrance, 7 – dendrite, 8 – neuron soma, 9 – nucleus, 10 – auxiliary cells, 11 – capsule cavity, 12 – hair channel, 13 – capsule wall).

The angle of hair deflection from equilibrium increases with sound intensity and depends on its frequency. The high-frequency limit of sound up to 80 dB deflecting the hair by 1° is 200–400 Hz. The low-frequency resonance maximum for hairs is 125–180 Hz, whereby they deflect by 1° at about 64 dB. The resonance maximum for the lowest frequency hairs is below 100 Hz; at 100 Hz, such hairs deflect 1° at less than 55 dB.

Low-frequency acoustic vibrations causing hair deflection give rise to undamped electric oscillations (microphone effect), which coincide with hair vibration frequency. The amplitude of these oscillations does not have a clear threshold; it gradually depends on sound intensity. Upon exposure to a sinusoidal sound of 80 dB at 100–150 Hz, the amplitude of the electric oscillations of the receptor repeating the shape of the sound wave is 300–350 μV. At the same sound intensity but with the frequency raised to 300–350 Hz, the amplitude of continuous electric oscillations taken from the receptor declines by 2.5 times (Fig. 41). The amplitude of action potentials generated by the neuron to threshold deflections of the hair (about 1°) is 765 ± 31 μV.

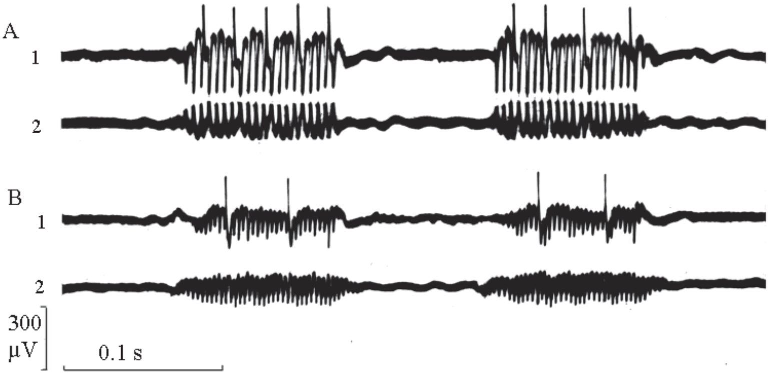

Fig. 41. Electric responses of (1) fast-adapting trichoid sensillae to sound pulses (2) of two different frequencies (A, B).

Distinctions in the resonance properties of hairs provide a frequency discrimination of signals of equal intensity and duration. Receptors with hairs intermediate in their resonance properties between high and low-frequency ones react to raising the frequency of a 100 ms sound impact from 150 to 350 Hz by decreasing the number of pulses in the response from 7.20 ± 0.41 to 2.20 ± 0.20. Sound intensity is encoded in the number and repetition rate of neuronal pulsing.

The receptor's adaptation time is connected to the intensity and temporal structure of the sound stimulus. To a continuous sound of 200–300 Hz at 75 dB, the receptor adapts in 0.6–1.0 s. With the intensity increasing to 85 dB, the adaptation time doubles or triples. The adaptation takes longer upon changing a continuous sound stimulus to a pulsed one. The recovery rate and the duration of the latent period in the phonoreceptor's response depend inversely on sound intensity (Fig. 42).

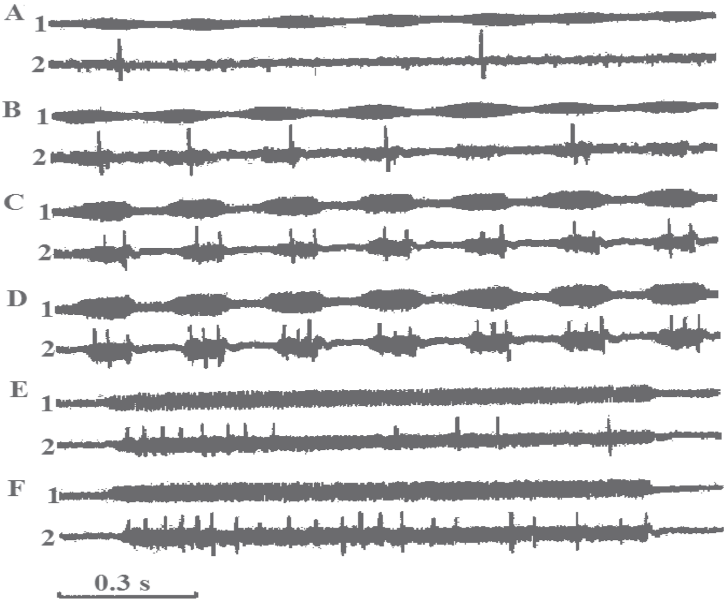

Fig. 42. Electro-responses of a rapidly adapting trichoid sensilla (2) to sound vibrations (1) differing in duration and intensity: A – 70 dB, B – 73 dB; C, D – 78 dB; E, F– 83 dB.

Upon hair deflection, the neuron generates spikes; their number depends on the angular velocity and the final angle of hair deflection (Fig. 43). Upon a 15–20° deflection at some 100°/s, the receptor generates 9.0 ± 0.5 pulses. When the deflection increases to 30–40°, the pulse number grows to 20.0 ± 1.1. No spontaneous firing has been observed. The fixation of the hair in any position differing from the initial position does not stimulate the nerve cell into spiking (Eskov, 1975), as is typical of the bee gravireceptor (Thurm, 1963) and wind sensitive hairs of the locust (Svidersky, 1980).

Fig. 43. Spikes of the sensilla neuron upon hair movement: 1 – deflection from equilibrium position; 2 – return to initial position.

Perception of substrate vibrations. In the honey bee, as in many other insects, the receptors of substrate vibrations are subgenual organs, discovered by N. McIndo in the early twentieth century (McIndo, 1922). This organ is localized in the proximal part of the tibia, directly under the joint. The sensitive cells (scolopidia) of the subgenual organ combine the functions of receptors and transducers of substrate vibrations. Scolopidia are attached at two points – the top and the side; the free edge opposite the side attachment opens like a fan under gravity. The presence of freely suspended cells augments the effect of their deformation (stretching and compression) under the action of inertial forces (Fig. 44).

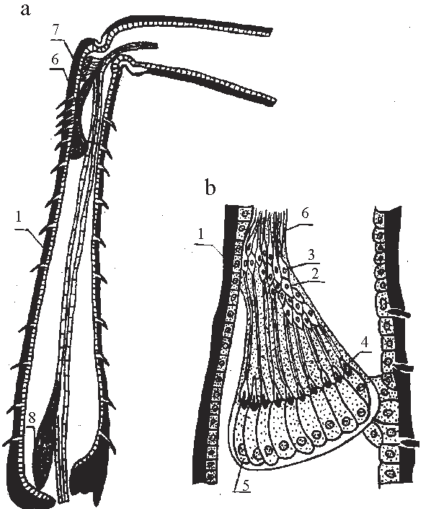

Fig. 44. Placement in the tibia (a) and the microstructure (b) of the subgenual organ of the honey bee that perceives the vibrations of the supporting substrate: 1 – cuticle, 2 – sensitive cells, 3 – their nuclei, 4 – scolopoid body, 5 – covering cell, 6 – axons, 7 – nerve fibers, 8 – a group of scolopoid cells that form a cordon organ that reacts to leg deformities (by: McIndo, 1922).

The subgenual organs of the honey bee are most sensitive to vibration in the 1–3 kHz range. According to data from electrophysiological tests, the maximal sensitivity is at 2.5 kHz (Autrum, Schneider, 1948), where an electric response was registered at a substrate vibration amplitude of 13 µm, while at 400 Hz it required vibrations of 250–2,000 µm. This, however, is inconsistent with the data of ethological studies. Bees on a solid substrate react with impeded motility to vibrations of 500–1,000 Hz at a minimal amplitude of 50 µm (Little, 1962). The perception of low-frequency EF involves specialized mechanoreceptors and/or unspecific structures. One of the mechanisms of EF perception is based on the attraction or repulsion, under the action of Coulomb forces, of primary mechanoreceptor transducers. Low-frequency EFs cause a vibration of fast-adapting trichoid sensillae and antennae. These vibrations are apparently perceived by Johnston's organs.

Fast-adapting trichoid sensillae. The amplitude of hair vibrations is in direct dependence on field intensity (Eskov, Mironov, 1989). In an EF of 50 V/cm, the hair deflects by 0.2 ± 0.1°, at 200 V/cm by 0.8 ± 0.1° and at 500 V/cm by 4.9 ± 0.3°. Changing the EF frequency from 1 to 3,000 Hz at the same intensity weakly influences the hair vibration amplitude. It declines only 1.2–1.5 times upon raising the frequency of electric oscillations from 10–80 to 500 Hz. Some hairs exhibit a weak resonance maximum at 10–80 Hz.

The hair vibration amplitude is influenced by the angle between the field intensity vector and the longitudinal axis of the hair. At 500 V/cm in an EF of 20 Hz, the amplitude of hair deflection reaches a maximum when this angle is 45°. When decreasing the angle to 0°, the vibration amplitude decreases by 40%, while at 90° the vibrations are completely dampened. However, an approach of the bee's head to one of the electrodes is accompanied by an increase of the hair vibration amplitude. For example, in an EF of 20 Hz at 550 V/cm, decreasing the distance between the electrode and hair tip from 1.30 to 0.13 mm increases the vibration amplitude about threefold.

The minimal intensity of a uniform EF deflecting the hair by 1° is 200–250 V/cm (Eskov, Mironov, 1989), which is necessary and sufficient to excite the receptor (Eskov, 1975). With an increasing EF and no uniformity, caused e.g. by the approach of two or more bees, the hair deflection grows at the same intensity (Eskov, Mironov, 1990; Eskov, Sapojnikov, 1976, 1979). Therefore, the threshold of sensitivity to EF for a bee's head on approach declines. For two bees in trophallaxis, it reaches 70–100 V/cm.

Along with low-frequency EFs, mechanoreceptors may be subjected to a strong influence of electrostatic processes. The hairs are attracted to a dielectric carrying a charge of any sign (Eskov, 1992). Bees remove antennae from an approaching charged body; the opposite takes place only in dead or anesthetized bees. The bee body's charge, in its turn, influences the doubling of the frequency of antenna vibrations under EF action. In accordance with increasing charge, the amplitude of antenna deflection grows.

Induced currents. A nonspecific mechanism of EF perception is based on stimulation by induced currents flowing at the sites of bees' contact with each other and/or with conducting surfaces (Eskov, 1992; Eskov, Sapojnikov, 1979). The frequency characteristic of EF sensitivity is mainly determined by the magnitude of the current induced thereby and the effectiveness of its contact action. Inasmuch as the currents induced in body covers are marked by high resistance, the current is nonlinearly related to EF frequency. Most probably, the induced current becomes maximal at a frequency of about 500 Hz, which is consistent with the region of highest sensitivity to EF. Its decline with frequency is favored by the reduction of sensitivity to current. Therefore, at frequencies exceeding 500 Hz, the field perception threshold grows sharply. High current sensitivity below 500 Hz determined the relatively low threshold for EF (Fig. 45). In a constant EF, no current is induced. Therefore, bees do not react to a DC EF.

The rather high variability of sensitivity to EF is connected with a multitude of random factors. These include the variability of contact with current magnitude. It largely depends on the conductivity of contacting surfaces, which is determined by their properties and state. At the sites of contact, there may be substances of varied conductivity: food, dust particles, wax, propolis, etc. In addition, with an increasing total mass of aggregating bees, the induced current grows.

The zones of bee crowding develop significant local gradients of intensity, which may be regarded as secondary fields, acting as additional stimulants (Eskov, Mironov, 1990; Eskov, 1992). For this reason, mutual stinging takes place in large aggregations. Aggressiveness is raised owing to the presence of conducting bodies in the EF. The stinging reflex is stimulated by an isolated conductor to which just a few tens of volts are applied.

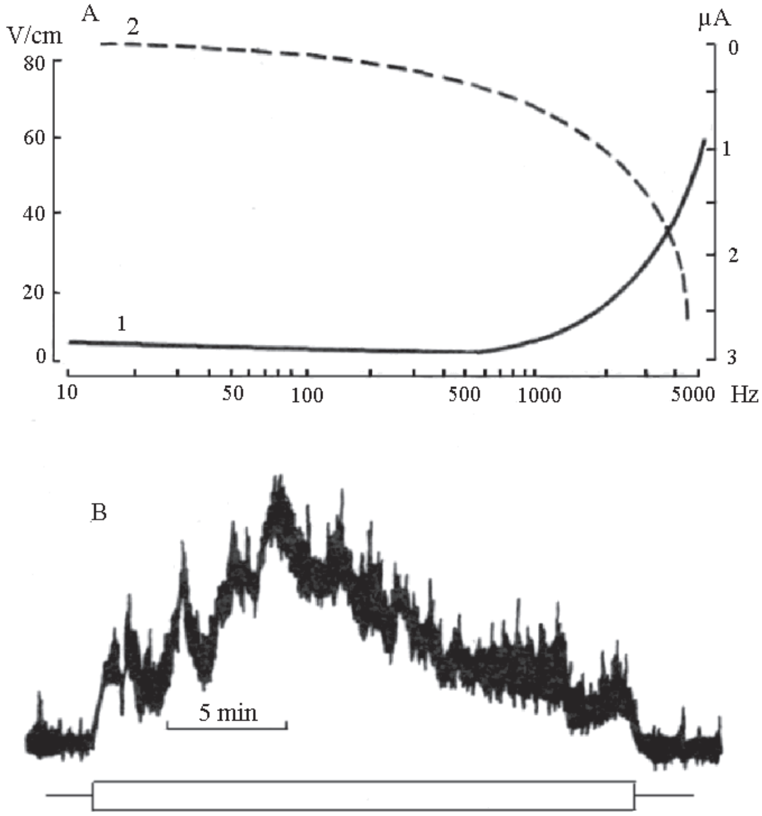

Fig. 45. Sensitivity to the EP of an electric current passing through a bee filling the goiter with a 50% sucrose solution: A – the frequency response of sensitivity to the EP (1); B – the change in the intensity of bee colony sounds in the band of 400–500 Hz to the effect of an EF of 500 Hz frequency at a voltage of 15 V/cm.

Antennae. Like a mechanical electrometer, the antennae of bees are attracted to an electrostatically charged body. When the head of a dead or anesthetized bee is moved towards a charged body, the angle between the antennae decreases (the antennae move closer together). Live bees turn their antennae away from charged bodies, acting more intensely when the body is closer or its charge is greater. In contrast, an uncharged body moves closer to the head of a bee when it is touched with the antennae.

The antennae vibrate in a low-frequency electric field. Antennal vibrations of the maximum amplitude are stimulated in an uncharged bee by an electric field with a frequency of approximately 50 Hz. When the bee’s body is charged, regardless of the sign of the charge, twice as high a frequency of an electric field is required to cause the greatest deflection of the antennae. The pattern is similar to the frequency dependence of oscillations that arise in an uncharged or charged conductor in an electric field.

The amplitude of antennal vibrations depends on the electric field’s intensity. At the resonance frequency (approximately 50 Hz), an antenna of an uncharged bee deflects, on average, by 3.0 ± 0.3° at a field intensity of 700 V/cm, 6.0 ± 0.5° at 950 V/cm, 12.0 ± 0.9° at 1200 V/cm, and 18.0 ± 1.6° at 1350 V/cm. A charge present on the bee’s body increases the antennal deflection in an electric field with the same frequency and intensity. In an electric field of 950 V/cm, antennae deflect by approximately 3.0 ± 0.2°, 10.0 ± 1.1°, 15.0 ± 1.6°, or 18.0 ± 1.7° when the body charge is 150, 400, 800, or 800 pC, respectively.

Antennal vibrations elicited by changes in electric field intensity are utilized by foraging bees to localize a dancing bee. A static charge on the body of a dancing bee waggling its abdomen is associated with the generation of an alternating electric field around the dancer. The field intensity in the space around the dancer changes at the frequency of abdominal waggling, which is approximately 14 Hz regardless of the target (food, water, or a new nest). The constancy of the abdominal waggling frequency distinguishes the dancer from numerous other active bees.

The shape of electrical oscillations recorded with a probe 3–5 mm away from a dancer’s abdomen depends on the probe’s position relative to the abdomen. When the probe is at a lateral side of the abdomen, each period of abdominal waggling is accompanied by a single change in the electric field’s intensity. A frequency of electrical oscillations that is two times higher is observed when the probe is at the distal part of the abdomen or over the abdomen.

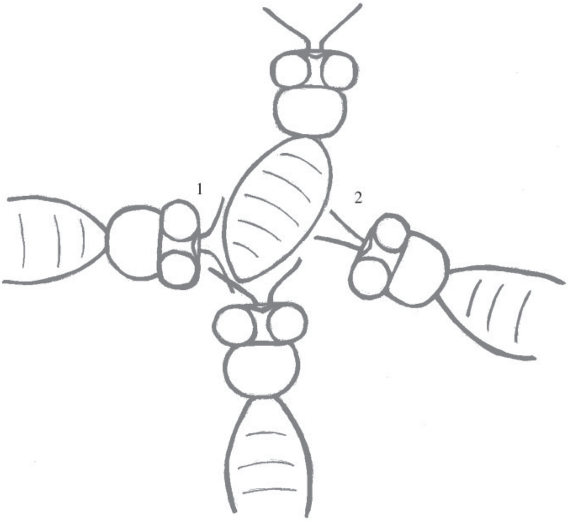

Fig. 46. The orientation of the antennae in the phases when the dancer's abdomen becomes (1) closer or (2) more distant.

The antennae of foraging bees mobilized by a dancing bee vibrate in response to periodical fluctuations in the intensity of the electrostatic field generated by the charged abdomen of the dancer. The vector and direction of antennal deflection in bees surrounding the dancer lack an ordered pattern and depend on the location of the bee among foragers (Fig. 46). The antennae of the foragers that follow the dancer deflect from a certain steady state two times per period of abdominal waggling movements. A single deflection of the antennae is elicited in bees located laterally to the waggling abdomen. The antennal deflection angle can vary substantially depending on the relative arrangement of the longitudinal axes of the dancer and forager bodies. The greater the angle is between the longitudinal axes of the bodies, the more asynchronous the antennal deflections are in terms of their deflection angles.

Trophic mobilization

In numerous observations performed by different researchers in the second half of the twentieth century, it was proved that information about the distance and direction to the discovered food source is conveyed by bees in the process of dancing. The form of the stereotyped movements of the dancers on the substrate depends on the distance to the indicated target.

But the information on its coordinates is conveyed only in the part of the dance that is attended by pendular motions (waggling) of the abdomen in the lateral plane. All the other phases of the dance do not have signal significance. Therefore the dancer bee tends to abridge these transitory (accessory) elements of the motive cycle. When informing about targets that are at a large distance, instead of making circular transitions from a completing to a new waggling phase, the dancer bee only shifts a few millimeters aside on a rectilinear trajectory.

The quantity of dancer bees in the house and their activity depends on the physiological state of the bee colony and the sufficiency of food stores. Also significant is the productivity of the feeding plots, which usually changes over the spring-summer season. In the periods when bees acquire new feeding plots, the dancers' activity grows. Each of them may be surrounded by 1 to 11 bees. As the foraging bees master the new feeding plot, their interest in signalers declines and their activity abates. In such situations, bees may appear to announce new sources of food or other flight errands. These signalers attract little, if any, attention from forager bees. The latter are again attracted by dancers as the productivity of the known feeding plot declines.

Signal stopping the dancer. The dancer bee stops from time to time, and then flies away to the known food source. In the pauses between dancing cycles it comes into tactile and trophic contacts with surrounding bees. During trophic contacts, the bees touch the dancer with their antennae and receive information on the odor of the food. Information on its quality is necessary for the mobilized bees in order to make a choice between several food sources found by scouts. This allows the time and energy of the foragers providing for the colony to be saved. Other conditions being equal, the bees mobilized by several signalers use the information from the one that has found a source of food with high carbohydrate content, being at that close to the hive.

In periods of high activity of the dancers, the bees often stop them by means of a specific sound signal (Fig. 47). The latter is a pulse of 185 ± 90 ms duration. The initial 25 ± 10 ms pulses of the signal have two intense components at 200–400 and 500–580 Hz, the first being some 10 dB louder. During the signal, the frequency of the first band shifts from 200–330 to 250–400 Hz (Eskov, 1976, 1977). Usually, a single such signal suffices to stop the dancer. Having stopped, it comes into trophic contact with one or several bees around it.

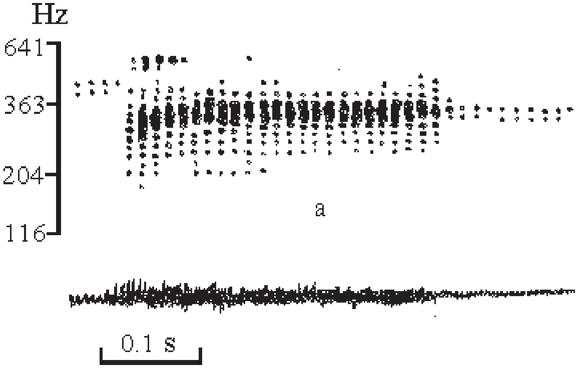

Fig. 47. The signal stopping the bee dancer.

Sound signal of flight distance. In the phases of the dance attended by waggling, the dancer generates sound signals. However, the waggling of the abdomen is not related to the sound generation mechanism (Eskov, 1969). The pulse rate in the signal exceeds the waggle frequency by 2.3–2.5 times. The sound pulses are not connected with any definite phase of waggling (Esch, 1962; Wenner, 1962a, 1962b).

Signal structure. The sounds generated by dancers are trains of pulses ordered in amplitude and succession rate. Each consists on average of four periods; the pulses are some 15 ms long, with pauses of much the same duration. The repetition rate is about 33 Hz.

The signal duration and the number of pulses therein are in direct dependence on the distance covered by the scout from the food source to the hive; this indicates that bees can measure the lengths of the path they cover. This has been proved by experiments in which labeled scouts visiting a feeder at a certain distance from the hive were subjected to a 15–18% truncation of one front wing. Thereby, immediately after truncation, the length of the sound signal upon returning from the source 200 m away from the hive grew on average from 609 ± 11 to 906 ± 16 ms, and the pulse number from 18.0 ± 0.5° to 21.1 ± 0.5°. Further, the bees adapted to the change of wing length, which was seen as a ~20% reduction of honey stomach filling, and the signal duration was adjusted to 864 ± 19 ms (Eskov, 1972a, 1972d, 1972c, 1979).

Race specificity. The duration of the dancer sound signal and the pulse number therein are among the racial traits of bees. For example, in yellow Italian bees signaling a food source 200 m from the hive, the 635 ± 13 ms signal contains 21.2 ± 0.4 pulses. Gray Krajina bees in the same conditions generate 12.9 ± 0.3 pulses over 434 ± 19 ms (Fig. 48).

Despite the fact that the honey bee adapts to changes in the biological situation inherently quickly and elaborates complex conditioned reflexes, the acoustic signaling of dancers is marked by high constancy. This is indicated by the preservation of a race-typical structure of distance signals when Italian bees are grown from egg to imago and later live in Krajina bee colonies. Likewise, the signal structure did not change in Krajina bees constantly living in an Italian bee colony (Eskov, 1971). Hence it follows that the generation and use of distance signals belong to the unconditioned reflex system of communication, excluding the non-hereditary transfer of experience.

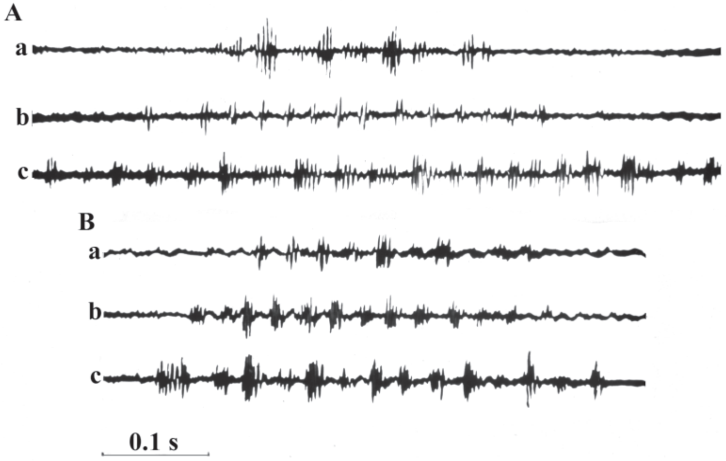

Fig. 48. The distance signals of the Italian (A) and Krajina (B) bees, announcing feed sources located at a distance of 30 m (a), 100 (b) and 200 m (c) from the hive.

Information component. Regardless of the tight connection between the distance to targets indicated by dancers and the length of signals, their structure shows substantial age-related variability. In the signals of old bees (40–50 days), the pulse's duration may vary from 15 to 150 ms; pauses are reduced accordingly (Fig. 49). Nonetheless, altered pulsing does not affect the relation of the signal length with the target distance. Hence it follows that the pulse structure and repetition rate do not have essential signal significance.

The apparent informative value of the acoustic signals of dancers, however, for a long time lacked experimental proof. After establishing the signal length–distance connection, attempts have been made more than once to mobilize foragers into directed flight using dancer models, which reproduced the postures and pulsed sounds corresponding to target indication. But the bees did not fly to the indicated target (Esch, 1967; Lopatina, Chesnokova, 1967). This is explained, on the one hand, by the fact that these tests did not reproduce the entire complex of signal means used by the dancers, first of all its EF. On the other hand, the dancers and the mobilized bees established a feedback system, based on their motivation to take part in supplying food with minimal energy expenditures.

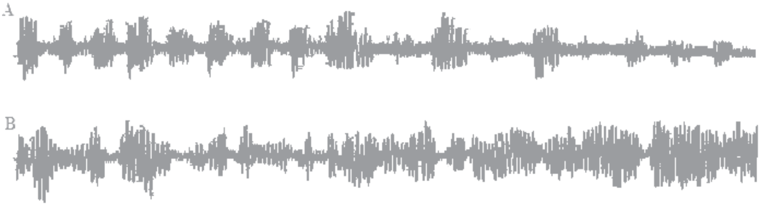

Fig. 49. Range signals generated by Italian bees, aged 27–28 (A) and 48–49 days (B).

The signal role of the sounds generated by dancers was proved in experiments with dancers and mobilized foragers in an observation hive (Eskov, 1972, 1979). Labeled dancers visited feeders with sucrose solution at a distance of 150 m from the hive. The natural interaction of the dancers and mobilized bees was supplemented only by extending the dancer signal with a playback of its last 200 ms. The extended signal was perceived by some 20% of bees that had been attracted by the dancer, so one fifth of mobilized bees overflew the feeder by 50 m (seeking it at 200 m from the hive).

Noise immunity. The diversity of biological processes in the bee colony is attended by sound generation. The most intense sounds are associated with the active airing of the nest, wherefore the bees beat their wings. The frequency of these sounds, which vary from 90 to 160 Hz, is in direct dependence on the temperature and carbon dioxide content in the hive. The second spectral maximum associated with heat production by bees is within 210–290 Hz. Upon the excitation of bees, components at 450–500 Hz are intensified (Eskov, 1972).

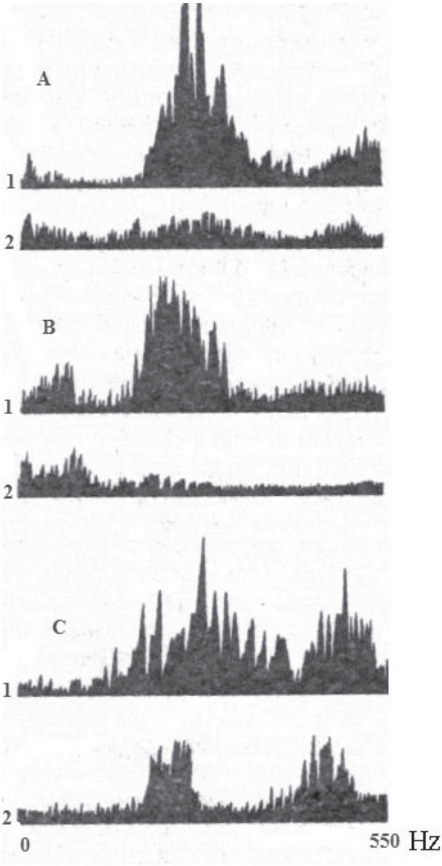

Fig. 50. The change in the spectral structure of the range signals (1) under the influence of the natural sounds of the bee colony (2): A, B – weak interferences; C – intense interferences.

Signaler bees adjust the frequency of their sounds under the influence of noises, depending on their intensity and spectral structure (Eskov, 1972). The ranges of signals and noises largely coincide, while the intensities differ by up to 15–17 dB. A decrease in intensity difference is attended by an increase in the frequency of the dancer-generated signal. At a signal/noise ratio of 3–6 dB, the signal frequency rises by some 50 Hz. Generally, the signals are shifted into the spectral regions with the least noise (Fig. 50). The reliability of the communication between the dancers and the mobilized bees is also improved by multiple repetitions of the signal.

Sociotomy

The propagation and spreading of the honey bee take place by means of the separation from a colony of a part (about half) of adult workers with a fertilized or virgin female (queen). This is the distinction between the propagation of separate individuals forming colonies and the multiplication of the colonies themselves (sociotomy), producing autonomous biological units individually subject to the action of natural selection and other factors of evolution.

Preparation for sociotomy may take time, ranging from several days to several weeks. In this period the bees preparing to leave the mother colony usually do not partake in trophic provision for the colony in building combs, feeding larvae, etc., but some of them (nest scouts) search for new settling places. Since the propagation instinct is coupled with the resettlement instinct, nest scouts search for new sites at various distances and, other conditions being equal, prefer the more remote ones (Seeley, Morse, 1978; Eskov, 1992; Camazine et al., 1999). The coincidence of the time of partition of many bee colonies and the limited number of suitable new locations entails competition between nest scouts from different colonies (Rangel et al., 2010).

Communication signals of queens. Honey bees are inherently monogynous. However, in the period of sociotomy, the colony raises several queens, of which only one remains. Others at different stages of development die from stinging by rivals. Usually, the first queens, having reached the imago stage, tend to eliminate those in sealed queen cells.

To detect and locate queens competing before sociotomy, they use specific acoustic signals (singing). Each signal includes 6 to 20 pulses 0.25–1.5 s long. The longest is usually the first one, or sometimes the second or third. Pauses are 0.15–0.25 s. The frequency of the intense components may change within a pulse as well as among pulses (Fig. 51).

The signals of calling queens always differ in their spectral structure. Thus, if the intense components of the song of one queen are within 350–450 Hz, the maximal energy in the response of a rival falls at 230–300 Hz, or vice versa. If two calling queens are joined by a third one, in the latter song the main components shift to higher frequencies than those of the former two.

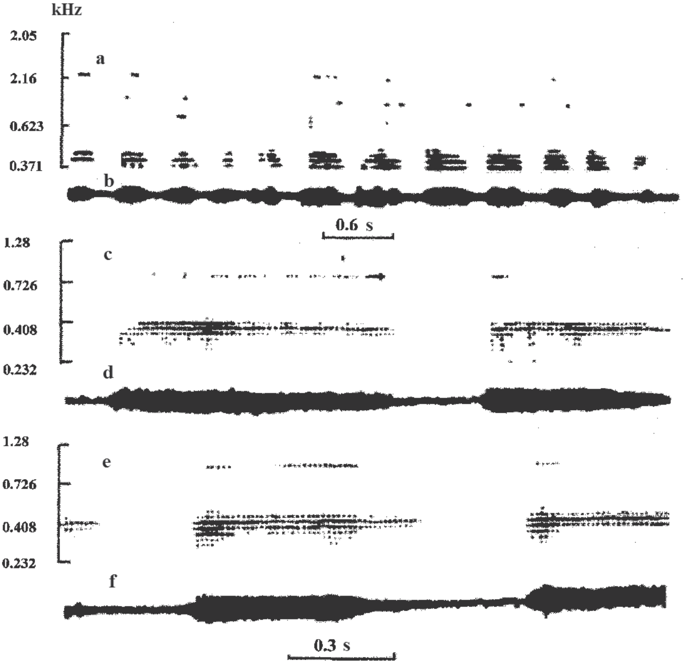

Fig. 51. Sound signal communication of (singing) queens: a, b, d – dynamic spectra; b, d, e – oscillograms; a, b – full cycle; c, d – the beginning; d, e – continuation.

The energy maxima in a queen song may be influenced by interference in the same range. For example, significant spectral alterations are caused by varying the intensity of noise focused at 185–225 Hz. A partial spectral overlap is admissible at a signal/noise ratio of no less than 7–8 dB so that the signal mainly fits into the range 210–290 Hz. When the ratio decreases to 2–3 dB, the signal peak shifts to 283–366 Hz. If the noise approaches or exceeds the signal intensity, the signal shifts more than 100 Hz upward of the initial one.

A singing queen always presses its thorax onto the substrate. Therefore, the communication signals of the queen are clearly registered by vibrosensors in contact with the combs where the calling queens are. The amplitude and frequency of comb vibrations fall into the region of the sensitivity of the vibroreceptors (subgenual organs) of the honey bee (Autrum, Schneider, 1948; Little, 1962; Eskov, 1992). For the queens themselves, it is not the air-borne vibrations that are significant but those propagating via the substrate (Eskov, 1973).

The signal role of sounds generated by queens is confirmed by the fact that they respond by singing to a playback or a simulation of their songs (Eskov, 1973, 1992). In simulations, the most effective are pure tones of 600 to 2000 Hz; it is important that their temporal structure should correspond to that of the queen song. However, simulated signals are less effective than natural ones. The queens usually respond to 1–3 playbacks of their song, but only to 7–9 full cycles of simulation.

Queens and worker bees react differently to a playback of a song via a vibroemitter contacting the comb. Queens get excited and head to the contact site. In contrast, workers stop abruptly (freeze) at every pulse of the queen song; they react in the same way to comb vibrations at 1000 ± 500 Hz (Eskov, 1973). The suppression of bee motility during the queen song favors the attenuation of acoustic noise and an unhindered approach.

Coordination of exit of the leaving colony. The bee colony that has differentiated into active and passive groups in the preparatory period, with surplus queens available, is ready to split into two colonies. One usually stays in the nest with young queens while the other leaves for a new settlement. Its flight is preceded by a sharp elevation of bee motility, stimulated by a progressively increasing group of dancing bees, which are the nest scouts. Like food scouts, they perform stereotyped motions, waggle and generate pulsed sounds; one distinction is that the nest scouts move over the combs very quickly between the dancing cycles.

Apparently, the main stimulus to mass exit comes from tactile interactions with hyperactive nest scouts and their acoustic signals (Eskov, 1976). These signals are irregularly delivered pulses 400–500 ms long. Each comprises three ranges of intense components. The first band at 300–450 Hz within some 150 ms shifts to 450–600 Hz and stays as such until the end. The second band appears with a delay of 15–25 ms relative to the first, and in about ~150 ms shifts from 720–870 to 900–1000 Hz. The third band appears in 20–30 ms from the onset of the signal and within another 20–60 ms shifts from 1050–1150 to 1150–1300 Hz (Fig. 52).

By the moment of mass flight, bee activity becomes maximal. Most of the bees agitated by nest scouts move to the exit from the nest and generate sounds peaking at 150–220 and 370–490 Hz, the former of which are 4.0 ± 0.3 dB louder. The flight of the queen is stimulated by specifically contacting bees which, approaching the queen for ~5 s, generate a sound with a frequency declining from ~500 to 200 Hz. The queen leaves the hive in the stream of exiting bees.

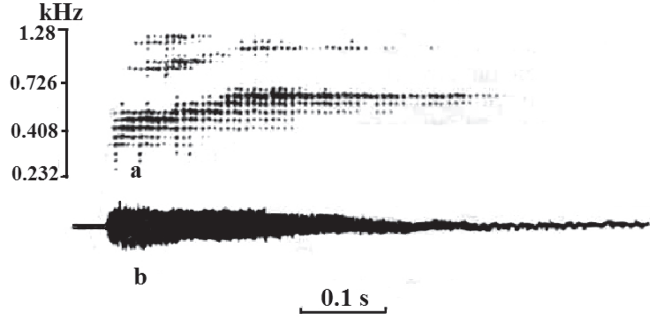

Fig. 52. Signal generated by quartermaster bees after selecting a settlement location.

Signaling of bees in a temporary cluster. Having left the mother colony, bees swarm for some time and then gather nearby on a tree branch or another support. The sound of a flying swarm is marked by two intense bands at 225 ± 25 and 470 ± 30 Hz, the first of which is 6.5 ± 0.9 dB louder. Near a swarm of ~15 of these bees, the sound intensity reaches 85 dB. However, these sounds are most probably unrelated to temporary clustering. The bees gather around the queen, having been guided by its pheromone (Eskov, 1992).

The bee cluster near the mother nest is a temporary stop before flying to the new settlement. Its duration may vary broadly – from a few minutes to a few days under the open sky; this depends on the time needed to choose the location for settling.

After clustering, the nest scouts fly away to visit the earlier marked places. Upon their return to the cluster, they notify other scouts about the location of their variants. The choice of the best one is taken by a comparison after visits by different scouts. They are able to assess the quality of the potential dwelling and its remoteness from the mother colony. This is suggested by the fact that, if a choice is available, bees prefer more remote dwellings (tree hollows or artificial constructs) of 30–80 L capacity (Seeley, Morse, 1978; Petrov, 1983; Eskov, 1992).

Nest scouts, like food scouts, signalize the target location by dancing, but their dances are distinguished by a longer duration; moving from one side of the cluster to another, the scout may dance for more than 5 min. However, the structure of acoustic signals and their relation with the distance to the target are the same. In particular, in food scouts dancing on the cluster (which happens when resettlement is delayed), upon visiting a food source 10 m away the signal duration is 256 ± 12 ms, at 50 m it is

278 ± 31 ms, and at 200 m it is 571 ± 34 ms, whereas in nest scouts the signals last 242 ± 34, 269 ± 36, and 564 ± 39 ms respectively.

Usually, approximately 40 min before the flight of bees delayed in clustering, the nest scouts returning from the finally chosen location begin generating specific sound signals lasting about 550 ms. The energy maximum therein changes with time: initially 300–550 Hz, in the next 50–100 ms it shifts to 500–780 Hz; the ending is usually marked by a lowering of the frequency (Fig. 53). This signaling before mass flight prevents the loss of bees that were busy with foraging and scouting.

Then, 3–5 min before take-off, a large part of the bees in the temporary cluster begin generating sounds with three main components at 175 ± 35, 350 ± 35, and 525 ± 35 Hz; the first band is 0.9 and 2.5 dB more intense than the latter two, respectively. The signalers move quickly over the cluster surface and beat their wings, which agitates all bees, and they start flying (Eskov, 1972, 1976).

The swarm heads toward the place chosen by the nest scouts, which show the way. The presence of a queen in the swarm is checked by its pheromone. If the queen stops flying, all bees gather around it within a few minutes. After some time, the bees attempt to continue the flight, using typical signal behavior. After multiple attempts, the bees start making combs at this random place, which most often does not fit their biological needs. Such a migrating colony is therefore doomed.

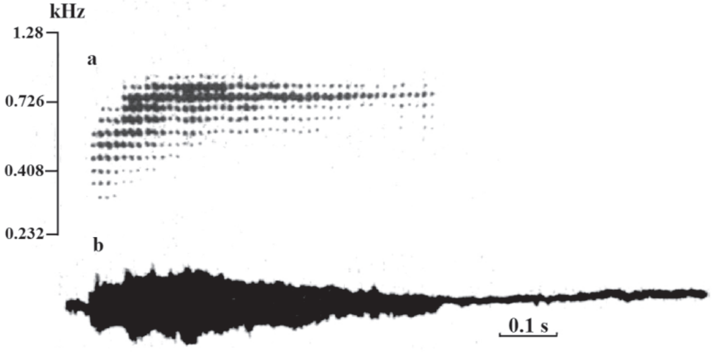

Fig. 53. The signal that stimulates the cessation of all the bees of the migrating colony (both quartermasters and foragers): a – dynamic spectrum; b – amplitude-time structure.

Related sound processes

The sounds that accompany some processes of the life activity of bees in some biological situations can stimulate specific responses. A certain synchronization in frequency is characteristic of the sounds generated by large aggregations of bees or their colonies.

Nest-Aerating Bees. Nest aeration by flapping wings is characteristic of many species of social hymenopterans. This reaction can be stimulated by an overheating of the nest or an increased content of carbon dioxide or water vapor in it. The bees that flap their wings (fanning bees) contribute to the intensification of air exchange between the space in the nest and the external environment (Himmer, 1932; Neuhaus, Wohlgemuth, 1960; Eskov, 1970, 1979, 2016).

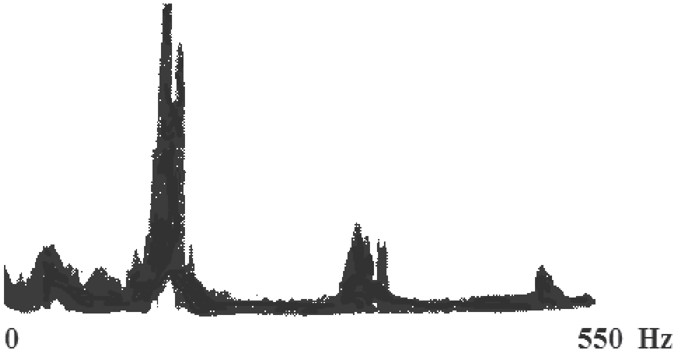

Fig. 54. The frequency-amplitude spectrum of the sound generated by the flapping of the wings of a bee ventilator.

The wing-flapping frequency and the sounds associated with this process in fanning bees vary from 75 to 155 Hz depending on the environmental situation. In particular, bees flap their wings at a frequency of 75–85 Hz at 35–35.5 °C and 145–155 Hz at 41–42 °C (Eskov, 1992). In the frequency-amplitude spectrum of the fanning bees, intensity components with frequencies that correspond to the wing-flapping frequencies can be distinguished, whereas the lower-intensity components are represented by harmonics (Fig. 54).

The generation of sounds by fanning bees with simultaneous airflow blowing stimulates a similar reaction in bees outside the nest (e.g. at the entrance to the hive). For this purpose, it is sufficient to bring a tube blowing a stream of warm air and to reproduce the sound generated by a fanning bee to the head of a bee in a passive state (Neuhans, Wohlgemuth, 1960). The signal role of the sound is confirmed by the fact that pure tones only have a high stimulatory efficiency in the range of 140–160 Hz. A frequency decrease to 105 Hz or an increase to 220 Hz reduces the probability of the response of passive bees by approximately five times.

Therefore, the sound that accompanies nest aeration is perceived by the worker bees as a signal indicating that the climate in the nest does not correspond to the biological requirements of the colonies. This is an example of a convergent similarity with the signaling system used by leaf-cutting ants (*Atta cephalotes*) trapped under a layer of soil (Markl, 1968). The similarity consists of the fact that both bees and ants, under favorable conditions, show protective responses after receiving a signal from individuals in danger. Bees contribute to the optimization of the microclimate in the nest, and ants dig for individuals in distress.

Acoustic noise produced by a bee colony. Various life activities of bees are accompanied by the generation of sounds with an intensity and frequency structure that depend on the physiological state of bee colonies and the environmental situation. In temperate and cold climatic zones, the acoustic noise produced by bee colonies reaches maximal intensity in spring and summer and is minimal in autumn and winter, varying in the range of 30–45 dB (Eskov, 1970).

Of the environmental factors, the temperature has the strongest effect on the intensity of the acoustic noise of a bee colony. In spring and summer, diurnal fluctuations in the ambient temperature of 10–15 °C are accompanied by changes of 5–6 dB in the noise intensity, which is usually higher at noon than at night. The exceptions are periods during which a foraging area with high productivity allows the active replenishment of food resources. In such cases, bees continue to aerate the nest actively, thus intensifying water evaporation from the nectar that fills the honeycomb cells.

The frequency-amplitude spectrum of noise is represented primarily by low-frequency components in the range of 70–500 Hz. The high-frequency components, which differ from the most intense low-frequency components by 40 dB, do not exceed 8,000–12,000 Hz.

The low-frequency components of the noise spectrum of a bee colony are represented primarily by the sounds generated by fanning bees. The energy maximum of these sounds varies in the range of 70–180 Hz depending on the activity of the bees involved in nest aeration. The second energy maximum is observed mainly in the range of 230–390 Hz. A drastic activation of bees leads to an enhancement of the components in the range of 420–550 Hz.

The frequency position of the energy maxima in each of the three ranges of intensity components of the spectrum of noises produced by a bee colony varies depending on the physiological state of bees and their locomotor activity. The position of the intensity peak in the first range of intensity components shifts from 75–85 to 145–155 Hz when the temperature in the center of the nest increases from 35.0–35.5 to 36.0–36.3 °C. This is accompanied by an increase in the noise intensity by 10–13 dB. A drastic increase in the carbon dioxide concentration in the nest has a similar effect on the intensity and frequency of the first-range components.

The intensity and position of the energy maximum in the second frequency range depend on the bee colony's state. In spring and summer, when the activity of foraging bees increases, the intensity peak of the second range shifts to the high-frequency region. The suppression of flight activity in cooling periods is reflected in the shift of the intensity peak to the low-frequency region.

Specific changes in the frequency and amplitude characteristics in the second range of intensity components are observed when bee colonies prepare for sociotomy, which is accompanied by the accumulation of individuals that are preparing for separation from the parent colony. These bees differ from the individuals that remain in the parent colony by their decreased locomotor activity. Therefore, the low-frequency region of the second range is enhanced. In the latter, 3–5 days before the swarm's departure, the low-frequency components in the range of 200–240 Hz increase relative to the adjacent fairly high-frequency ones (250–340 Hz) by 8–12 dB (Fig. 55). As a result, under conditions that are favorable for flights and food resource replenishment, the second range of intensity components is broadened or differentiated into two frequency ranges that differ in intensity. The intensity of low-frequency components of the second range of noise usually increases as the day of the departure of the swarm approaches. After the completion of sociotomy, only one peak of intensity components remains in the second range, the frequency position of which varies depending on the changes in the activity of bees (Eskov, 1970, 1979).

The components of the third frequency range are sometimes enhanced in highly excited bees. Many times during the spring and summer season, the components in the range of 400–550 Hz are enhanced immediately before the simultaneous flights of large groups of bees out of the hive. Before and during mass scale flights, the intensity of these components is close to the intensity of the second-range components. Similar changes in the noise spectrum of colonies are observed before and during swarming.

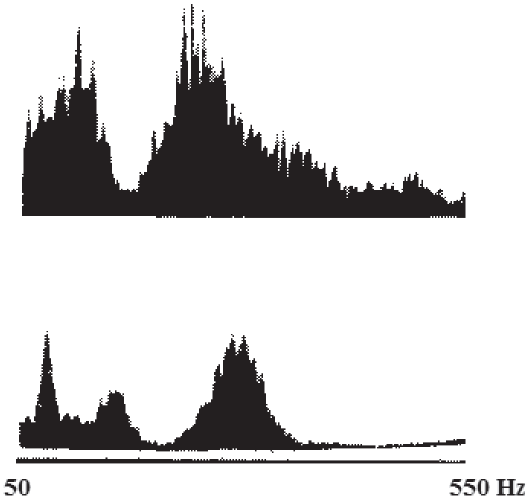

Fig. 55. Changes in the frequency-amplitude spectra of bee colony sounds in the process of sociotomy: A – 4–5 days before the exit of the swarm; B – 3 days after swarming (B).

Significant changes in the noise spectrum are induced by exposure to a low-frequency electric field. Bees exhibit maximum sensitivity to an electric field with a frequency of 450 ± 50 Hz. At an electric field intensity of 20–35 kV/m, the peak frequency in the second range increases by 25–35 Hz for 3–5 min, and its intensity increases by 4.5 ± 0.5 dB. This is accompanied by an enhancement of the third-range components of 12–15 dB.

Thus, the frequency-amplitude spectrum of the noise produced by bee colonies has a certain consistent pattern. Some shifts in the energy maxima in three ranges of intensity components of the noise spectrum of bee colonies depend on the physiological state of the worker bees, the activity of which strongly depends on the changes in environmental conditions.

Honeycomb cell vibrations. The maximum spectral energy of honeycomb cell vibrations in the spring and summer period is in the range of 80–165 Hz. The decrease in the honeycomb's vibration intensity from the energy maximum to the high-frequency region is 10–18 dB per octave (Fig. 56).

The most intense low-frequency vibrations of honeycomb cells are observed under the influence of acoustic streams generated by fanning bees. The frequency and intensity of these vibrations increases as the temperature rises above the value that is optimal for the bee colony.

During sociotomy, vibrations generated by conflicting queens are spread over honeycomb cells. High-frequency vibrations of a relatively low intensity are generated by the mechanical impacts of adult individuals on the honeycomb cells.

Developing individuals make a small contribution to the vibrations of honeycomb cells. Honeycomb vibrations with a disordered temporal structure occur when young bees gnaw the honeycomb cell caps.

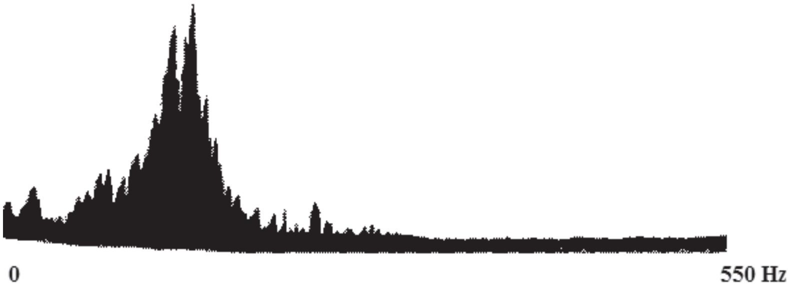

Fig. 56. The spectrum of vibrations of honeycombs in the spring-summer period at 25–28 °C.

Slight vibrations are generated by individuals at the larval stage of development. They periodically turn over in the honeycomb cells, which is accompanied by the generation of vibrations from the friction of the larval body against cell walls. Larval locomotion is stimulated by starvation. The intensity of the sounds generated by the older larvae near the surface reaches 12–17 dB, which is insufficient for their perception through the air. However, the honeycomb vibrations generated by the larvae rubbing against the cell walls can be perceived by the subgenual organs of adult bees. This may attract the nurse bees to the hungry larvae. A similar type of signal communication between larvae and adults is used in communications of paper wasps (Eskov, 1979). They generate vibrations by rubbing their mandibles over the cell walls. There is a parallelism in the use of acoustic communication between larvae and adults in remote hymenopteran species – bees and wasps.

Conclusion

The honey bee exploits a multichannel communication system provided mainly by using multifunctional organs for signal generation and perception. The generation of specific sound signals is an additional function of the flight apparatus. This mechanism, based on the vibration of thoracic segments by indirect flight muscles, allows the bee to broadly vary the spectral structure of the signal as it is delivered. The body covers can accumulate an electrostatic charge, thereby generating an electric field.

Low-frequency acoustic and electric fields are perceived by fast-adapting trichoid sensillae between the compound eyes and the occipital suture. The frequency, intensity, and duration of a signal leading to hair vibrations are coded by the bipolar neuron of the receptor into the number and repetition rate of action potentials. The latter are generated by the neuron only while the hair moves (deflects). The hair fixed in a deflected position does not give rise to action potentials and provides no response to electric or acoustic fields (Eskov, 1975). This property makes the function of the honey bee's trichoid sensillae basically different from that of known tactile and wind sensitive (Rozhkova, Polishchuk, 1978; Swidersky, 1980) as well as gravity-sensitive organs (Thurm, 1963).

Intra-nest signaling includes acoustic communication via the air and substrates. Signals that are mainly air-borne are received by trichoid sensillae; substrate vibrations are perceived by subgenual organs. The match of signal transmission and perception mechanisms obviates energy losses in transitions between different media, thus facilitating the communication.

Signaling via substrates is typical only of queens competing in the period of sociotomy. Queens, like other bees, generate sounds by their flight apparatus, but differ from others in that, during signaling, they press their vibrating thorax to the substrate (comb). These vibrations affect the queens and the worker bees differently. Queens get excited and go up the intensity gradient to meet their rival, while workers freeze, which favors the unhindered approach of the queens. In addition, queens generate signals with major components in noise-free ranges.

The reliability of the communication between the singing queens increases due to the fact that the locomotion of worker bees is suspended (for the time of singing). This allows the competing queens to meet, which is required for the termination of the period of uncertainty, which ends with the survival of only one of them. The latter, after mating and the beginning of ovipositioning, restores the physiological homeostasis of the colony, which had been disturbed by the sociotomy.

In the bee colony phylogeny, the specific response of the worker bees to the honeycomb vibrations generated by the queen's singing probably developed in view of the fact that the bees moving on the path of approaching queens would hamper their interaction. Natural selection favored the acquisition of the instinct of suspended locomotion by worker bees in response to a queen singing. This was probably achieved by the aggressive attitude of queens to the moving bees, which were attacked similarly to the competing queens.

The instinct of the worker bees to respond by suspended locomotion to the honeycomb vibrations, the temporal and frequency structures of which correspond to the singing of queens, has been sealed so strongly that it is realized even in situations that are not related to sociotomy. Bees always respond with suspended locomotion to the simulation of a queen's singing transmitted over honeycomb cells. However, bees are activated by unordered vibrations of the nest structure that are associated with nest destruction or penetration by enemies and robbers (Eskov, Toboev, 2011).

The air acoustic channel is used in the communication system of the bee dancers with the bees mobilized by them. The relatively low sound intensity of the dancers is obviously necessary and sufficient to ensure reliable communication. This contributes to the correction of the frequency-amplitude spectra of the sound signals of dancers under the influence of interference. It is probable that the increased impact of the sound vibrations of the air on the hairs of the phonoreceptors of the mobilized bees is achieved by the generating of an EF by a dancer via the electrification of her body.

The ability of bees to estimate distance and memorize and reproduce the coordinates of the target (food, water, new settlement) during the dance is unrelated to the transfer of experience by training/learning. The entire system of communication in the honey bee colony is implemented by complex instincts, the realization of which is stimulated by bees' needs that vary depending on their physiological state and ecological situation. In species phylogeny, acoustic and other forms of communication promoted the consolidation and transformation of the bee colony into a biological unit.

The broad ecological valence of the honey bee, acquired in conjunction with the perfection of thermal adaptation (Eskov, 1995a), is in great measure associated with the development of an efficient, reliable, and economical communication system. The involvement of only separate bees in the search for and notifications about food sources saves the colony energy that is spent on food provision. Natural selection also favored the acquisition of the instinct for finding resettlement sites and notifying the colony of their coordinates during sociotomy. The energy expenditures of a group of nest scouts are negligible relative to the losses that might be suffered if the whole moving colony had to look for a new home.

The phases of the development of acoustic signaling in the communications of forager bees can be traced in the species that are at different levels of social organization. In relatively primitive bees (e.g. in trigonines) that find a food source, the duration of mobilization acoustic signals is not associated with its distance from the nest. In *Trigona scaptotrigona*, signals with a frequency of 300–500 Hz are pulses with a duration of 0.5–2 s. These signals stimulate forager bees to move to the beehive entrance. The mobilized bees determine the direction to the food source from the flight trajectory of the signaling bees (Essh et al., 1965). In more advanced stingless bees, e.g. meliponines, the duration of the signals produced by the forager bees depends on the distance to the food source. As the distance to the source increases from 100 to 600 m, the signal duration in forager *Melipona quadrifasciata* increases from 0.5 to 0.75 s (Esch, 1967).

In trigonines and meliponines, signaling forager bees randomly move over the nest and activate the passive bees in it. In honey bees, sound is generated when a bee moving along a straight trajectory with a vector that is determined by the direction to the intended goal waggles its abdomen in the lateral plane. However, this abdominal waggling has nothing to do with the sound generation mechanism (Eskov 1969, 1979).

The bees mobilized by the dancing bee distinguish it by the abdominal waggling from the numerous adult individuals on honeycomb cells and control the distance required for receiving the message by means of the electrostatic field (Eskov, 2003, 2013). Its generation is associated with the accumulation of an electrostatic charge on the dancer’s body, the value of which is approximately two orders of magnitude greater than the charge of passive bees. The presence of a charge on the body and abdominal waggling lead to a change in the static field’s intensity around the signaling bee.

The localization of an electrified dancer by the bees mobilized by it is achieved by the attraction of antennae and trichoid sensillae, performing the function of phonoreceptors. They react with the generation of nerve impulses on the deviation of hairs when approaching and removing a charged abdomen. Along with this, trichoid sensillae synchronously vibrate at a frequency of about 250 Hz, responding to the shrinking action potentials on the vibrations of hairs that occur under the action of pulsating sounds and electrical oscillations generated by statically charged wings. The synchronous effect on the trichoid sensillae of acoustic and electrical oscillations provides for an increase in the reliability of the bee dancer's transmission of information about the distance of the flight target.

The dancer's movement along a straight path, in that phase of the dance when it waves its belly, is required by the mobilized bees to determine the direction of the goal of the flight. Using Johnston's antennae organs and specific trichoid sensillae located at the articulations of the head with the chest and the chest with the abdomen, performing the gravitational function (Little, 1962), the mobilized bees determine the angle of deviation of their own body relative to the gravity vector, and along it the direction of movement of the dancer.

The high variability of the oscillations of the antennae in amplitude and frequency excludes their use to control the distance to the flight target for the mobilized bees. The sensitivity of the Johnston organs is insufficient to respond to the weak sound and the accompanying electrical oscillations generated by the dancers. The role of antennae is limited to the localization of a charged signalman and maintaining the distance necessary for the bees to be mobilized for the perception of information about the coordinates of the flight target.

The acoustic interaction between the dancing bees and the bees mobilized by them developed during the transformation of a bee colony into an evolving biological unit. In this sense, a bee colony is a sort of complex multicellular organism, in which the mobile information of receptor organs is encoded in action potentials. In a bee colony, a similar function is played by the acoustic communication signals which are used at an unconditioned reflex level. For this reason, bees are indifferent to such signals under conditions that are inadequate for their realization in a bee colony. The coding of acoustic information by a dancing bee into a sequence of acoustic pulses and their effect on the mobilized bees shows a convergent similarity with the coding of sensory stimuli perceived by receptors of organisms of different complexities into a sequence of action potentials.

A bee colony which unites many biologically independent individuals has a single regulatory center. The colony is consolidated by the hormones secreted by the queen and the interaction of worker bees. They play the dominant role in an adequate response to changes in environmental conditions. The discrete structure of the frequency-amplitude spectrum of the acoustic noise produced by a bee colony is apparently generated by the processes of the mutual adjustment of the sounds generated by different groups of bees in similar states. This is a manifestation of the generally consistent pattern of the auto-synchronization of the processes occurring in biological systems (Wiener, 1961) that do not have a single regulatory center. However, the synchronization of acoustic processes in a bee colony helps to unite its members into a single biological system representing an evolving biological unit.

CHAPTER FIVE

ANOMALIES IN BEES' BEHAVIOR

Working individuals in bee colonies are characterized by a high orderliness of behavior. Anomalies of the bees' behavior occur under the influence of factors that are not typical for their life. These factors more often than not have an anthropogenic origin.

The anthropogenic factors that destabilize the behavior of bees include the electric fields of high-voltage power transmission lines (HV PTL) and pollutants spoiling the food areas of bees.

Effects of the HV PTL

The HV PTL generates a significant local increase in the intensity of low-frequency electric fields (EF). Most often, a current with a frequency of 50 or 60 Hz is transmitted via the HV PTL. The strength of the electric field in the area of the HV PTL depends on the voltage applied to them. At an altitude of 2 m from the ground, the intensity of the electric field under the PTL 500 kV is on average 6 kV/m, under the PTL 750 kV it is ≈11 kV/m, and under the PTL 1500 kV it is ≈17 kV/m. With other things being equal, the intensity of the EF depends on the sagging of the wires and the relief.

The sensitivity of bee colonies to low-frequency EF. The minimum intensity of the EF stimulating the initial phases of bees' activation depends on their number. A small colony, containing 2 ± 0.2 thousand bees, responds by changing the intensity of the spectral structure of sounds at industrial frequency EF (50–60 Hz) at a voltage of 0.9 ± 0.3 kV/m. A colony containing 11 ± 1 thousand bees is excited under the action of an EF of the intensity of 0.6 ± 0.2 kV/m.

Since the perception of the EF is mainly associated with the bees' irritation with induced currents (Eskov, 1992), their effectiveness is influenced by many random factors. These include the frequency of tactile contacts between the bees, their orientation in relation to the EF lines, the electrical conductivity of the supporting substrate, etc.

Sealing in the bee entrance with bee glue. The specific means used by bees to protect the bee entrance against irritation with induced currents include covering it with wax and bee glue. At the end of the summer, bees usually reduce the entrance by covering its inner surface with bee glue and wax. Natural selection favored the development of this instinct since a decrease in the entrance size makes it possible to reduce heat loss when the external temperature decreases during the fall-winter period. But in the summer, bee colonies need oxygen in the nest. Therefore, the sealing of the hive entrance with bee glue, which protects the bees from the irritation of currents, limits the saturation of the nesting space with oxygen and prevents cooling when the hive is overheated in the summer.

The amplification of induced currents when approaching the HV PTL stimulates bees to isolate the entrance. For example, in the beehives located at a distance of 50–55 m from a 500 kV HV PTL, bees cover the bee entrance with 1.1 ± 0.2 g of bee glue, at a distance of 12 m – 12 ± 1.4 mg, and under the extreme phase of the line – 37 ± 3.2 mg (Eskov, Bragin, 1986; Eskov, 1992).

There are cases of complete bee entrance embedding by colonies that lived during the summer period in hives that were under the HV PTL. Bee colonies that found themselves in such conditions soon died (Warnke, Paul, 1975).

Summer activity and the mass of the honey crops. In the hives that were under the HV PTL of 500 kV, the frequency of departures and arrivals of bees varied widely. With no apparent reason, large groups of bees flew out of the hives and circled at a distance of several meters from them. Then the mass flights-out of bees stopped for some time.

Bees flying out were characterized by increased aggressiveness. They attacked and stung all objects moving near the hives.

Bees in the hive are irritated by the induced currents that are flowing in the process of tactile contacts with each other and with conductive surfaces. An increase in humidity or in the intensity of an EF increases irritation by induced current.

The EF of the HV PTL influenced the filling of honey crops in bees flying out of hives and returning to them. With a low productivity of the forage area, the bees flying out of the hives under the power transmission lines had a crop content of 2.3 ± 0.4 mg, and at a distance of 50–55 m from the line – 1.5 ± 0.2 mg.

An increase in the productivity of the forage area influenced an increase in the filling of crops in bees that are in the zone with the influence of the EF of the HV PTL and remote from this field. The bees that flew out of the hives that were under the PTL had a crop content mass of 2.7 ± 0.6 mg, and at a distance of 50–55 m from the line – 2.2 ± 0.4 mg.

The abundant nectar secretion significantly influenced the increase in the filling of crops in bees returning to the hives. For bees that were under the power lines, the filling of crops was 31.6 ± 2.8 mg, and at a distance of 50–55 m – 49.6 ± 3.4 mg.

Intra-nesting microclimate. *Temperature*. The EF of the PTL activating the locomotion of bees effects an increase in intra-nesting temperature. When hives approach the PTL, the temperature inside them increases, which is associated with the irritation of bees by induced currents.

An uneven density of bees in the hive is associated with unequal temperature increases in different zones of the nesting space. Under the influence of the hive's movement under the 500 kV PTV, the temperature of the inter-cell space facing the entrance increased from 35.2 ± 0.5 °C to 39.9 ± 0.7 °C, and in the center of the nest – from 35.5 to 37.4 °C (Fig. 57A). On the periphery, multi-path decreases of temperatures to baseline levels and increases by 2–3 °C took place (Fig. 57B).

Daily fluctuations in temperature were expressed in its decrease in the evening hours when the bees were returning to the hive. This is due to the increase in the bees' number and tactile contacts between them.

After removing the hives from under the PTL, the temperature in them gradually normalizes. The temperature deviation from the initial values is most often expressed in its decreases at the periphery of the nest.

CO_2 and O_2 concentration. The EF violates the implementation of bees' hereditary program of regulation of the nesting microclimate by exciting them. The increase in locomotor activity and the intensification of metabolism increases oxygen consumption and the release of CO_2. Therefore, in the nests of colonies placed under the HV PTL, a relatively high level of CO_2 concentration is maintained, exceeding its normal value by 2–6 times.

The daily dynamics of the flight activity of bees is associated with a change in their numbers in the nest. This effects a change in population density and tactile interaction. Under a PTL, this increases irritation to induced currents. Therefore, in the center of the nest, from the evening until the morning, the CO_2 content reaches a maximum value, and by the middle of the day, it is reduced to a minimum.

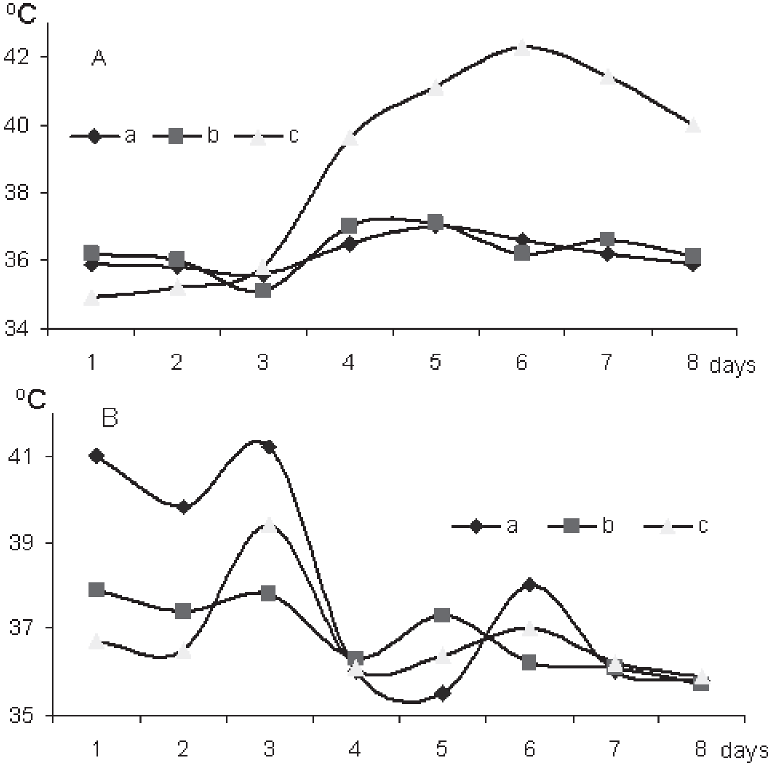

Fig. 57. Temperatures in the bee entrance (a), at the center (b) and on the periphery of the nest (c) of the colony when moved under the 500 kV PTL (A) and 50 m away from it (B).

From morning to midday, the concentration of CO_2 increases dramatically in inter-cell spaces from the entrance side. For example, in the morning, in the center of the nest placed under a 500 kV power line, the CO_2 concentration reached 1.8%, and in the pre-entrance area – 1.1%. By the middle of the day, in the center of the nest, the CO_2 concentration decreased by 2.3 times, and at the entrance it increased by 1.5 times.

In the nest of the colony located 55 m from the power lines, the concentration of CO_2 in the nest center in the morning was at 0.5%, while in the middle of the day it had increased to 0.9%, and in the pre-entrance zone, it was at 0.3 and 0.2%, respectively.

Critical levels of pollutant accumulation by bees' bodies

In recent years, a specific form of the death of bee colonies has been observed everywhere, which takes place predominantly at the end of the summer and/or in the fall (Dainat et al., 2012; Ilyasov et al., 2017). Bee colonies leave their nests, leaving their brood and food reserves behind (Eskov, 1992; Eskov, Eskova, 2018).

In urbanized territories, areas near highways are widely used for keeping temporary or permanent bee colonies. The emissions of motor vehicles and the friction of moving cars' tires on asphalt are associated with soil and air pollution by toxic substances (Elshin, 1986), among which lead and cadmium pose the greatest threat to bees (Eskov and Eskova, 2019).

The sensitivity of bees to the contamination of feed with lead or cadmium. The influence of lead or cadmium content on the metabolism activity and the amount of feed consumed by bees kept in entomological cages has been established. The consumption of a pure 60% sucrose solution in terms of one bee averages 51 ± 4 μl/h. In the presence of lead in a carbohydrate solution of 20 mg/l, feed intake was halved, and at 50 mg/l, it decreased by about three times. Similar changes in feed intake occurred when 2 or 5 mg/l of cadmium was present.

According to the oxygen consumption, the activity of the bees' metabolism decreases in accordance with an increase in the contamination of feed. With a pure 60% sucrose solution, the consumption of oxygen by one bee is 18.1 ± 0.92 μl/min. Accordingly, the increase in lead content in the sucrose solution to 0.5, 2, 20, and 40 mg/l caused oxygen consumption to decrease on average to 17.1 ± 0.87, 9.86 ± 0.67, 1.66 ± 0.13 and 0.32 ± 0.05 μl/min (Fig. 58A). A similar downward trend in oxygen consumption, but at a lower level, was traced when the maximum cadmium content within the solution was increased to 0.25, 0.5, 2, and 5 mg/l (Fig. 58B).

In bees consuming pure solutions of sucrose, the respiratory coefficient (RC) was maintained at ~1. After consuming a 60% sucrose solution for 4–5 days in which the lead content is 500 mg/l, the bees migrate from the feeder and aggregate on the cage walls, forming a dense sedentary cluster. Bees that refuse to consume food live for 2–5 days at the expense of internal reserves, and the RC decreases to 0.79 ± 0.03. A similar change in the RC occurs in bees after stopping the consumption of the sucrose solution in which the cadmium content reaches 50 mg/l.

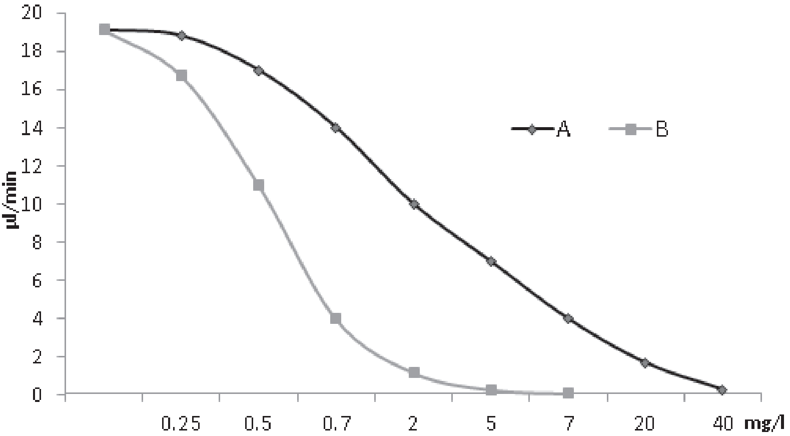

Fig. 58. Oxygen consumption in terms of one bee (µl/min) depending on the amount of lead (A) or cadmium (B) that is contained in a solution of 60% sucrose.

The accumulation of lead and cadmium in the body of bees consuming food contaminated by these elements. Bees that were originally in different anthropogenic pollution conditions consumed different amounts of feed. This has been established for bees whose colonies, being in different conditions, varied in honey pollution (Eskov, Eskova, 2018b, 2019). In particular, honey in the hives of colonies located near a busy highway contained 1.5 times more lead and 2.9 times more cadmium than in a slightly polluted park area, and 1.7 and 1.2 times more pollen, respectively.

The contamination of the feed consumed by different groups of bee colonies influences the lead and cadmium contamination of the bees' bodies. Compared to bees from the park, ones located near the highway exceeded them in lead accumulation in different parts of the body by about three times, and in the organs of the digestive tract – by two times (Table 8). In terms of cadmium accumulation, the body sections and organs of the digestive tract of these bees differed by about three times (Table 9).

Table 8. Accumulation of lead in bees' bodies (mg/kg), the feeding areas of which differ in their anthropogenic contamination before the beginning ($M1 \pm m1$) and after stopping the consumption ($M2 \pm m2$) of a 60% sucrose solution containing 0.5 g/l of this element.

Body parts and bee organs	Park area		Near the highway	
	$M_1 \pm m_1$	$M_2 \pm m_2$	$M_1 \pm m_1$	$M_2 \pm m_2$
Head	0.047 ± 0.015	1.713 ± 0.197	0.087 ± 0.009	1.845 ± 0.267
Thorax	0.068 ± 0.009	1.984 ± 0.261	0.302 ± 0.071	1.884 ± 0.068
Abdomen *	0.086 ± 0.016	3.464 ± 0.698	0.134 ± 0.019	3.603 ± 0.718
Crop	0.057 ± 0.005	1.557 ± 0.198	0.073 ± 0.023	1.646 ± 0.267
Stomach	0.113 ± 0.014	4.082 ± 0.628	0.247 ± 0.042	3.962 ± 0.752
Rectum	0.099 ± 0.008	8.823 ± 1.505	0.205 ± 0.019	10.44 ± 0.605

* without intestinal tract

Table 9. Accumulation of cadmium in bees' bodies (mg/kg), the feeding areas of which differ in their anthropogenic contamination before the beginning ($M1 \pm m1$) and after stopping the consumption ($M2 \pm m2$) of a 60% sucrose solution containing 50 mg/l of this element.

Body parts and bee organs	Park area		Near the highway	
	$M_1 \pm m_1$	$M_2 \pm m_2$	$M_1 \pm m_1$	$M_2 \pm m_2$
Head	0.0184 ± 0.0045	0.5463 ± 0.0492	0.0682 ± 0.0118	0.7781 ± 0.1075
Thorax	0.0463 ± 0.0091	0.3032 ± 0.0526	0.0905 ± 0.0126	0.2593 ± 0.0303
Abdomen*	0.0431 ± 0.0096	1.7229 ± 0.2744	0.1103 ± 0.0176	1.6874 ± 0.2679
Crop	0.0173 ± 0.0028	1.3422 ± 0.2924	0.1082 ± 0.0187	1.318 ± 0.0198
Stomach	0.0556 ± 0.0135	0.4449 ± 0.5959	0.1279 ± 0.0222	0.5032 ± 0.0064
Rectum	0.0583 ± 0.0134	2.0206 ± 0.3807	0.1712 ± 0.0321	2.266 ± 0.1971

* without crop, stomach and rectum

Regardless of the contamination of food consumed by bee colonies that were in different environmental conditions (near the highway or in the park area), the lowest accumulations of lead and cadmium varied in the head sections, and the largest – in the stomachs and rectums. In bees from colonies in the highway zone, lead accumulation in the rectums increased by 5.1 times, and in those from the park zone – by 5.6 times. In terms of the content of cadmium in these groups of bees, the rectums exceeded the head sections, respectively, by 2.9 and 3.7 times.

The consumption of food by bees placed in entomological cages depended on the conditions in which the bee colonies lived. Bees from colonies whose feeding areas adjoined the highway or were located in the park zone stopped consuming the sucrose solution with an admixture of 500 mg/l of lead after 5 and 4 days, respectively. The activity of feed consumption by each bee from colonies located on the highway was 4.2 ± 0.4 µl/h, and in the park area – 6.2 ± 0.5 µl/h.

The differences in food consumption by bees influenced the accumulation of lead in their bodies. For those bees whose colonies were near the highway, the lead content for 5 days increased in head sections by 21.1 times, in thoracic sections – by 6.2 times, in the abdominal sections – by 26.8 times, in crops – by 22.5 times, in stomachs – by 16.0 times, and in rectums – by 50.9 times ($P \geq 0.999$). In bees from the park area, these differences were significantly higher, amounting to 36.4, 29.2, 40.3, 27.3, 36.1 and 89.1 times ($P \geq 0.999$), respectively (Table 8).

Bees stopped the intake of feed which contained 50 mg/l of cadmium during the day. During this time, each bee from the colonies whose feeding areas were located near the highway consumed 4.8 ± 0.7 µl/h of feed, and from the park zone – 7.3 ± 1.1 µl/h. Accordingly, the content of cadmium in the head sections of bees increased by 11.4 times, in the thoracic sections – by 2.9 times, in the abdominal sections – by 15.3 times, in the crops – by 12.2 times, in the stomachs – by 3.9 times, and in rectums – by 13.2 times, or, for bees from the park zone, by 29.7, 6.5, 40.0, 77.6, 8.0 and 34.6 times ($P \geq 0.999$) (Table 9), respectively.

After the feed consumption, the activity of bees' locomotion and metabolism sharply decreased. In this state, they survived for 3–7 days by using up their internal reserves (Eskov, Eskova, 2018, 2019).

The behavior of bees leaving the hives in the fall-winter period. The unmotivated gathering of bee colonies kept in anthropogenically polluted territories occurs after the end of the spring-summer period, during which the bees are in an active state. In the passive period of life, which starts once the daytime temperature has been established at a level not exceeding 5–7 °C, the bees aggregate in the nesting space, and the queens' reproductive diapause occurs (Eskov, 2003).

The gatherings of bee colonies that take place during the passive period of their lives differ from the resettlement of large groups of bees during a sociotomy or of all adult individuals upon resettlement. The resettlement and sociotomy of bee colonies are preceded by a search for suitable places of settlement. All migrating bees fly out of the hive for a few minutes and gather not far from it, and then they fly away with the queens in the direction of the new home found by the quartermaster bees (see Chapter 4).

Bees leaving their nests during the passive period of their life fly out of the hives one by one at intervals ranging from a few seconds to minutes. Leaving bees do not stay at the entrance and do not make orientation flights. Their flights are similar to ones in the spring-summer period of forager bees to their known sources of food. However, unlike foragers, flying bees always start flying along a straight-line trajectory. The shading of the bee entrance by the board, which is attached obliquely to the front wall of the hives, does not suspend the gathering of bees that has begun. They do not fly around an obstacle, but linger in front of it and soon die. In December–January, up to 1,700–2,500 bees can gather between the board and the hive during the day. All of them soon fall into a chill coma and die.

In the anthropogenically polluted areas during the fall-winter period, there is often a gathering of individual colonies, and less often of the entire apiary. Gatherings of individual colonies occur during periods of fall-winter thaws at 0–5 °C. Gatherings of a large number or all colonies located in very polluted territories often occur at the very beginning of wintering – in October–November – at 3 to 7 °C (Eskov, Eskova, 2019).

Up to 14–21 kg of feed (almost a full winter stock) and from tens to several hundred dead bees remain in hives of colonies flying off in the fall. They form dense clusters on the comb. There can be from 70 to 110 worker bees. Regardless of the time of the bees' gathering, dead queens in the hives are not detected.

The content of lead and cadmium in food reserves and the bodies of dead bees remaining in the nests of gathering colonies. In honey remaining in the nests of colonies flying off in the fall-winter period, the lead and cadmium content can be up to 2.5 mg/kg and 0.3 mg/kg, respectively. The lead content in pollen sometimes reaches 21 mg/kg (Table 10).

Table 10. The content of lead and cadmium in the food reserves and the bodies of dead bees (mg/kg) remaining in the nests of gathered bee colonies.

Samples	Lead	Cadmium
Honey	2.41 ± 0.548	0.31 ± 0.031
Pollen	12.14 ± 0.242	0.29 ± 0.049
Pupae	7.74 ± 0.596	0.26 ± 0.023
The body parts of the bees:		
Head	1.6993 ± 0.4943	0.202 ± 0.044
Chest	6.4169 ± 0.612	0.312 ± 0.031
Abdomen	8.1142 ± 0.5207	0.398 ± 0.038

The pupae bodies located in the abandoned nests of colonies, in terms of the lead and cadmium content, occupy an intermediate position between the content of these elements in honey and pollen (Table 10), which corresponds to the carbohydrate and protein food consumption by adult bees of brood feeding.

The lead content reaches 5.4 ± 0.46 mg/kg, and cadmium – 0.304 ± 0.032 mg/kg in the bodies of dead adult bees. As in other environmental situations, these elements are unevenly distributed in the three parts of the bee's body. The highest lead and cadmium content is in the abdominal sections, and the smallest – in the head (Table 10).

Defeat of the bee colonies by varroatosis

Varroatosis is a disease of honey bee colonies caused by the acari *Varroa destructor*. Parasitized on an Indian bee (*Apis cerana indica*) in the second half of the twentieth century, this species of acari spread to the honey bee. The ranges of these species overlap in Southeast Asia. Having appeared on the honey bee, the tick quickly spread due to human participation in this bee species' wide area.

The reproduction of ticks takes place in the cells occupied by developing drones and worker bees. In the brood cells, the breeding ticks feed on the hemolymph of the pupae, which, with a large invasion, leads to developmental abnormalities and a decrease in the bees' and drones' viability (Sadov, 1978; Glinski, Yarosh, 1987).

At the end of the summer and the beginning of the fall, after the completion of the bees' brood reproduction, the female ticks leave the cells of the honeycomb and attach themselves to the bees' bodies. Using their mouthparts, ticks pierce thin areas in the bees' body cover, through which the hemolymph is sucked. Tick-secreted saliva inhibits lysozyme synthesis, weakening the humoral protection of the bees (Glinski, Yarosh, 1987).

Conditions of a warm climate and acquired ethological devices allow the Indian bee to withstand the expansion of the tick. With strong varroatosis, these bees leave their nests, leaving their brood and food reserves (Koeniger, 1987). Ants, penetrating into the abandoned nests of Indian bees, destroy the infected brood and the food. A nesting cavity cleaned by ants may be colonized by another moving colony of bees.

At the end of the summer and the beginning of the fall, the number of ticks increases dramatically when the honey bees' brood reproduction is completed. Ticks, localized on the bees, irritate them, which, with a high degree of infection of varroatosis, causes colonies to leave their homes. Leaving the nest, adult individuals, like the Indian bees, leave their brood, which has not completed its development, and feedstocks. However, unlike the Indian bees, the honey bees leaving their homes at the end of the summer and in the early fall have no chance of surviving. Colonies remaining in their dwellings also die from varroatosis and related diseases.

Colonies of honey bees, exposed to severe infection with varroatosis, leave the hives under weather conditions favorable for flights. After leaving the hive, the worker bees, together with the queens, gather at a short distance from it, forming a cluster similar to that of sociotomy. After some time, quartermaster bees, having found a suitable place to settle, carry the whole colony with them.

Having established a new place of settlement, bees begin to build honeycombs, but the lack of trophic support does not allow the bees to withstand the upcoming cooling. Bees soon die from chill coma and starvation.

The use of bee colonies contaminated with varroatosis for wintering prevents their gatherings. However, the wintering of colonies takes place with the increased activity of bees. This is indicated by the spectral structure of sounds generated by bees, which is not typical for wintering. The intensity and position of the energy maxima in the range of 200–400 Hz depend on the ratio between the number of ticks and bees. With the ratio of ticks and bees being 2–8 to 100, the energy maximum relative to the low-frequency region of the sound spectrum was at 246 ± 4 Hz, and the high-frequency one – at 343 ± 3 Hz. An increase in the ratio between the number of ticks and bees to 40–60 to 100 is accompanied by an increase in the frequency of energy maxima. The low-frequency energy maximum is shifted by 343 ± 3 Hz, and the high-frequency – by 369 ± 3

Hz. In this case, the amplification of the intensity of sounds does not occur (Eskov, 2004b).

The colonies’ condition and viability after the completion of wintering depend on their infection with varroatosis. Colonies with a relatively low number of ticks actively develop at the beginning of the spring-summer period. The weakening of colonies that were not subjected to the release of ticks occurs by the end of the summer and the beginning of the fall. Colonies with a high number of ticks can overwinter, but during the summer the number of worker bees in them sharply decreases. These colonies, before they begin wintering, usually die or fly off.

Conclusion

Factors stimulating bee behavior anomalies have different lethal efficiencies. A low probability of the death of bee colonies is possible when hives under are an HV PTL. Hives are often placed in glades, overgrown with honey-bearing vegetation. However, the placement of hives under an HV PTL destabilizes the behavior of bees. They are irritated by induced currents in contact with each other and conductive surfaces.

An increase in the nesting temperature influences the increase in energy expenditures by bee colonies and creates unfavorable conditions for the brood. For normal development, it needs a temperature which does not exceed 34–35 °C, and it sometimes reaches 40–41 °C in the nests of bee colonies under the 500 kV transmission line. Increasing the concentration of carbon dioxide increases the expenditures of bees on nest aeration and accelerates their physiological aging (Eskov, 1977).

Current irritation reduces the productivity of flying forager bees, which is reflected in a decrease in the amount of food stored by bee colonies during the spring-summer period. The increased aggressiveness of bees departing from the hives poses a threat to the beekeeper and visitors of the apiary.

An intensive coating of the bee entrance with bee glue provides bees arriving and departing from the hive with protection against blows induced by the current. However, high activity in blocking up the aisle passage can lead to the walling up of the hive and the subsequent death of the bee colony. This is characteristic of bees with a congenital increased tendency to an intensive filling of gaps in the dwelling and restriction of the bee entrance with bee glue and wax, which usually occurs in late summer and early fall.

Bee colonies that are under the HV PTL during the spring-summer period have a low probability of death by the beginning of the fall. However, the potential opportunity to overcome the long wintering decreases. Therefore, bee colonies should be placed at some distance from the power lines. Reducing the irritation by induced currents in bees in areas of power lines is achieved by placing them under the crown of trees or grounded iron roofs.

The lethal effect is completed in the fall-winter gatherings of bee colonies, whose forage areas are subjected to intensive pollution by lead and/or cadmium in the spring-summer period. The presence of these elements in carbohydrate feed has a repellent effect for bees. The threshold of sensitivity of bees to lead in a sucrose solution is ≈0.5 mg/l, and for cadmium it is ≈0.2 mg/l.

The consumption of contaminated feed by bees in cages or hives leads to the accumulation of pollutants in the body and, especially, in the part of the abdominal region that is occupied by the rectum. Reducing the consumption of contaminated feed does not allow bees (that are in cages or hives) to get freed from pollutants that accumulate along with excrement in the rectum. Bees are freed from excrement in the process of cleansing during flights, which is impossible when keeping bees in cages. In a temperate and cold climate, natural bee flights out from hives cease in the fall. From now on, they cannot be freed from excrement, and when consuming contaminated feed, the accumulation of pollutants in the body occurs.

For bees kept in cages, a refusal to consume feed and unmotivated fall-winter gatherings of bee colonies from hives occur with similar accumulations of lead and/or cadmium in the head and abdominal parts of the body. The critical level of lead accumulation in head sections, modifying the behavior of bees, is 1.6–1.8 mg/kg, and for abdominal sections – 8–10 mg/kg, and for cadmium – 0.5–0.6 and 2.0–2.3 mg/kg, respectively.

It is probable that the anomalies of the programmed and modified behavior of bees are mainly due to the accumulation of pollutants in the head sections. The increasing accumulation of lead and/or cadmium in rectums, when it is impossible to defecate, is ensured by an increase in these elements to critical levels in the head sections.

The content of pollutants in trophic substrates consumed by bees depends on the length of the period from the beginning of their passive life to the completion of unmotivated gatherings. The probability of their displacement from the fall to the winter increases with a decrease in lead and/or cadmium in the winter feed reserves and, accordingly, with an increase in the time required to achieve critical levels of the accumulation of these elements in the head and abdominal sections of bees.

Bee colonies, whose gathering at the end of the summer and beginning of the fall stimulates ticks to parasitize on bees, have a possibility for survival in subtropical and tropical conditions. This is taken advantage of by Indian bees, whose range is limited to warm climates. Following a strong varroatosis invasion, they fly to new places of settlement, freeing themselves from ticks located in the brood cells.

A colony of honey bees, when leaving a dwelling with brood in which ticks develop, is partially freed from the ticks. However, in conditions of temperate and cold climates, the individual supply of carbohydrate food, which bees fill their honey crops with before leaving the nest, is not enough to settle in a new place of settlement. In late summer to early fall, the flowering of melliferous vegetation usually ends. Therefore, resettled colonies, unable to prepare fodder stocks, die from hunger during the wintering period.

The prevention of the gathering of bee colonies whose feed stocks are contaminated with toxic chemical elements can be promoted by replacing a contaminated feed with a pure carbohydrate feed.

Various acaricidal preparations are used for varroatosis in beekeeping. They reduce the intensity of the invasion but are not safe for the bees themselves. Therefore, for the rehabilitation of bees in preparation for wintering, it is advisable to introduce substances with sorption properties, for example, chitosan, into carbohydrate food. The use of biologically active substances in feed, for example, dihydroquercetin, which is a natural flavonoid obtained from Dahurian larch wood, also contributes to the viability of bees (Eskov et al., 2013).

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