ISSN 1062-3590, Biology Bulletin, 2015, Vol. 42, No. 2, pp. 154–162. © Pleiades Publishing, Inc., 2015.
Original Russian Text © E.K. Es'kov, 2015, published in Izvestiya Akademii Nauk, Seriya Biologicheskaya, 2015, No. 2, pp. 197–206.

ECOLOGY

Ethological–Physiological Effects of Hypoxia on the Honeybee *Apis mellifera* L.

E. K. Es'kov

Russian State Agrarian Extramural University, ul Yu. Ficheka 1, Balashikha, Moscow oblast, 143900 Russia
e-mail: ekeskov@yandex.ru

Received February 7, 2014

Abstract—Information on the effect of hypoxia on the behavior and physiological state of the honeybee was compiled and systematized. It was shown that, in the course of colonization of temperate and cold climate zones by the honeybee, natural selection favored the acquisition of an effective mechanism of thermoregulation and high tolerance to hypoxia. It was noted that bees can develop under conditions when the CO_2 concentration exceeds the content of this gas in the surface layer of the Earth by more than three orders of magnitude; however, this leads to deviations in the morphometric traits from the norm. At the adult stage, anesthesia with carbon dioxide was found to reduce the body weight and the water content in it. It was shown that the effect of anesthesia in adult bees increases with temperature and that hypoxia in adult bees and queens accelerates their senescence and reduces viability.

DOI: 10.1134/S106235901502003X

INTRODUCTION

The honeybee exhibits a high tolerance to hypoxia, which is associated with its adaptation to life in shelters and especially with the ability to aggregate in response to cooling (Hess, 1926; Budel, 1955; Michener, 1974; Es'kov, 1995). 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 (Heinrech, 1985; Es'kov, 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 (Es'kov, 1995).

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

FACTORS AFFECTING THE AIR GAS COMPOSITION IN THE BEE HIVE

The changing components of the gas medium in the bee hive are mainly CO_2 and O_2. Oxygen is consumed, and CO_2 is released during the respiration of adults and developing bees.

Brood. At different stages of brood development, from the egg to the pupa, oxygen consumption varies widely. The main contribution to CO_2 accumulation in the hive is made by the brood (pupae 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 the middle age and increases by the end of development.

Regardless of the age and 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 that the response of pupae (table). In particular, when the temperature increases from 30 to 40°C, the oxygen consumption by 1- to 3-day-old larvae increases, on average, 1.6 times; and that by pupae, 1.9 times.

Adults. The metabolic activity of bees, other things being equal, depends on their age. Young bees consume a relatively small amount of O_2 in the brood area of the hive, where a stable temperature of approximately 35°C is maintained. At this temperature, 3-day-old bees consume, on average, 11 mm^3/min ($C_v = 30\%$) of O_2. Oxygen consumption is reduced 1.6 times at 40°C and 85 times at 0°C. In bees of the middle and older age groups, the highest O_2 consumption (56.6 mm^3/min ($C_v = 20\%$)) is observed at 30°C. At 0°C, it decreases 218 times; at 40°C, 1.7 times.

Queens responded to a temperature increase in the range that does not lead to the suppression of locomotion by intensification of respiration. Unlike the worker bees, the young (10-day-old) queens consumed the greatest amount of O_2 (on average, 25.4 mm^3/min ($C_v = 13\%$)) at 40°C. When the temperature decreased by 10

Dependence of the respiratory quotient (K_d) and O_2 consumption by larvae (29 ± 8 mg) and old pupae of worker bees on the gas medium composition and temperature

Temperature, °C	Oxygen content, %	K_d	O_2 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	2.31 ± 0.18	1.53 ± 0.12
	16.8 ± 2.1	1.43 ± 0.11	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

and 20°C, the O_2 consumption was reduced 1.7 and 1.9 times, respectively. The O_2 consumption by the 1- to 3-year-old ovulating queens under these conditions increased approximately twice.

Male bees in spring and summer are usually located on the periphery of the nest, where the temperature is 5–10°C lower than in the brood area. At 25°C, male bees consumed a relatively large amount of O_2 (66 mm^3/min (C_v = 22%)). When the temperature increases to 30 and 40°C, the O_2 consumption by them decreased 1.5 and 2.6 times, respectively.

The respiratory quotient (K_d) value depends on temperature. In worker bees, in the range of temperatures stimulating the cold torpor, $K_d > 1$. At 10°C, the mean K_d value was 1.3 (C_v = 8%); at 0°C, K_d = 1.6 (C_v = 6%); at 15–35°C, $K_d \approx 1$; and at 40 and 50°C, K_d = 0.85 (C_v = 7%) and 0.81 (C_v = 9%), respectively. In the queens under the age of 10 days, K_d at 30°C was 0.9 ± 0.05, whereas in the 1- to 2-year-old queens in the period of high reproductive activity, K_d was 0.7. In the male bees, in the range of optimal temperatures, K_d was approximately 1.

EFFECT OF HYPOXIA ON THE DEVELOPMENT OF BEES

In spring and summer, bees actively prevent the accumulation of high CO_2 concentrations in the hive. This determines the necessity of a separate study of the effect of hypoxia on bees developing in natural (hives) and artificial (thermostats with a specific gas medium) conditions.

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 location of the worker bee larvae 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 environmental 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 (C_v = 4%) and the body weight was 102 mg (C_v = 7%), whereas at 2.5% CO_2, these parameters were 4.80 mm (C_v = 8%) and 103.4 mg (C_v = 14%), 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, but caused no

changes in the size of the abdominal tergites (Es'kov and 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 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.

Under artificial conditions, the physiological state and viability of bees largely depends on the conditions of their development at the larval and pupal stages. Development under adverse conditions (in terms of temperature and forage area productivity) can lead to elimination of a considerable part of developing bees at the stage of eggs and young larvae, and the bees that survived to the adult stage are characterized by a reduced viability.

Metabolism activity. The larvae of worker bees at the optimum temperature for their development (33–35°C) responded to a decrease in the content of O_2 in the air from 19.5 to 6 ± 0.4% by a decrease in O_2 consumption by a factor of 13.9 ($P > 0.999$). The K_d 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 1.1 and 1.4 times, respectively (table).

Hypoxia had a long-term inhibitory effect on the metabolic activity. For example, the O_2 consumption by 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 (Es'kov and Es'kova, 2011).

The consumption of O_2 by pupae, similarly to larvae, decreased when its content in the air decreased (table). At the optimum temperature, a decrease in the O_2 concentration by 19.5–12.7% was accompanied by a decrease in the O_2 consumption by a factor of 6.2 ($P \geq 0.999$). 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% caused a 1.5-fold decrease in the O_2 consumption, whereas at the upper (37°C) boundary, the O_2 consumption increased 1.1–1.2 times. A decrease in the O_2 concentration in the gas medium from 19.5 to 16.1 and 12.7% led to an increase in the K_d value in pupae from 0.96 ± 0.08 to 1.59 ± 0.11 and 2.05 ± 0.18, respectively (Es'kov and Es'kova, 2011).

Brood viability. The effect of hypoxia on developing bees during incubation to the adult stage depends on the conditions under which the brood developed from the egg to the prepupal stage (cell sealing) in the nests of their families. All bees that developed to the prepupal stage under favorable conditions for their families showed a high tolerance to hypoxia. When such broods were incubated at a 5% CO_2 concentration, the death of bees did not exceed 0.1%. It increased significantly when the concentration of this gas increased, on average, to 10 and 15% and reached 14.4 ± 2.5 and 24.8 ± 4.1%, respectively.

The effectiveness of hypoxia increased when bees developed in their families to the pupal stage under adverse conditions—poor productivity of the fodder area and a temperature not higher than 20°C (Es'kov et al., 2013a). At 5 ± 1% CO_2 concentration, the elimination of such bees in the period from the prepupal to adult stage was approximately 1%. On average, 68 ± 2.7% of bees did not survive to the adult stage if the brood in the first 6 days was incubated in a gas medium containing 10% CO_2. However, when bees were kept under natural air conditions for the first 6 days and then transferred to a medium containing 10% CO_2, the mortality of bees decreased to 59 ± 2.6%. All bees died after incubation at 10–12% CO_2 throughout the period of development from the prepupal to adult stage at 34°C for 12 days.

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 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 regions was 11 ± 0.27 mg ($C_v = 12.3$%). The incubation of the brood at the optimum temperature caused an increase in the head weight, on average, by 3.9% at 5% CO_2, by 5.9% at 10% CO_2, and by 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. In the bees that developed to the prepupal stage under unfavorable conditions, the weight of the 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 the 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 body of the 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

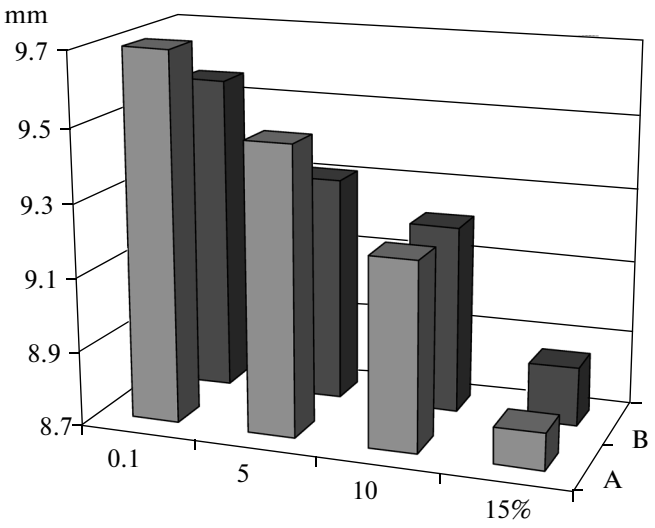

Fig. 1. Variation in the mean length (ordinate axis) of (A) left and (B) right wings of the bees that developed from the prepupal to adult stage at different CO_2 concentrations (abscissa axis).

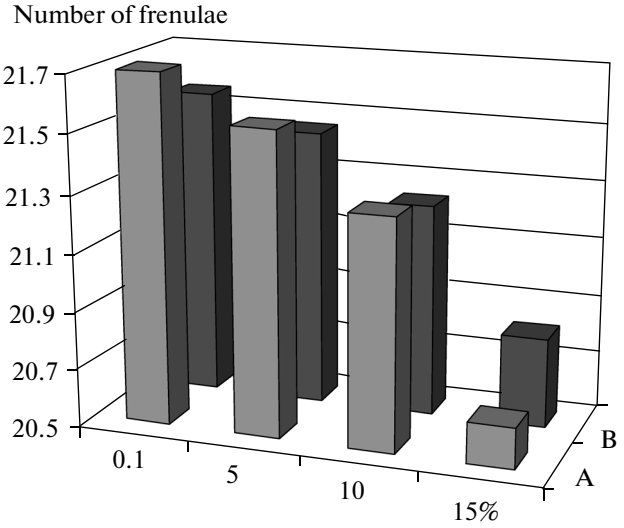

Fig. 2. Variation in the mean number of frenula on (A) left and (B) right wings of the bees that developed from the prepupal to adult stage at different CO_2 concentrations (abscissa axis).

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 ($C_v = 5.4\%$); in the thoracic regions, 72.1 ± 0.52 ($C_v = 3.7\%$); and in the abdominal regions, 83.8 ± 0.61 ($C_v = 4.1\%$).

Morphometric parameters. In bees that had no marked morphological abnormalities, the lengths of the proboscis and wings and the number of frenula decreased as the concentration of CO_2 in the gas medium 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% ($P > 0.99$). In the bees that developed at 0.1% CO_2 concentration, the proboscis length was 5.61 ± 0.084 mm ($C_v = 3.7\%$).

The length of the left and right wings (Fig. 1) and the number of frenula on the hindwings changed in different ways under the influence of elevated CO_2 concentrations. In the natural gas medium, the length of the left and right forewings was 9.74 ± 0.036 mm ($C_v = 2.1\%$) and 9.56 ± 0.021 mm ($C_v = 1.6\%$), 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 hindwing 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 hindwing decreased by 2.7, 3.4, and 7%, respectively (versus 6.76 ± 0.44 mm at 0.1% CO_2) ($P > 0.99$).

Similarly to the changes in the size of the left and right hindwings, the number of frenula on them showed a similar decreasing trend and an increased asymmetry as the CO_2 concentration increased (Fig. 2). At the specified increased values of CO_2 concentration, the number of frenula 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 in the bees that developed under hypoxic conditions was accompanied by an increase in the limits of their variability range (Fig. 3). 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 medium 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.

The increase in the CO_2 concentration was accompanied by a specific variation in the proboscis length. Normally, it varied from 4.7 to 6.3 mm (in approximately 70% of bees, it ranged within 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. In these bees, the distribution of proboscis lengths ranging from 4 to 6.2 mm had two peaks in the frequency of occurrence of 4.3–5.1 mm (~50%) and 5.5–5.8 mm (~30%). A similar distribu-

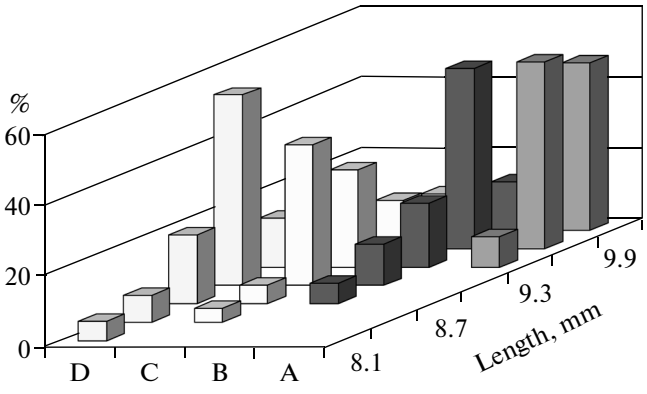

Fig. 3. Distribution of variation (ordinate axis) in the wing length in the bees that have developed from the prepupal to adult stage in (A) natural gas environment at (B) 5, (C) 10, and (D) 15% CO_2 concentration.

tion pattern with respect to the proboscis length was characteristic of the bees that develop at 15% CO_2 concentration (Fig. 2).

In the bees that developed to the prepupal stage under unfavorable conditions, after the completion of development in thermostats with free access of air, the length of the proboscis was 5.01 ± 0.08 ($C_v = 6\%$), the length of the left forewing was 9.31 ± 0.03 mm ($C_v = 2.1\%$), the length of the right forewing was 9.39 ± 0.04 ($C_v = 2.4\%$), the length of the left hindwing was 6.69 ± 0.03 ($C_v = 2.7$), and the length of the right hindwing was 6.70 ± 0.02 mm ($C_v = 2.2\%$). The number of frenula on them was 20.1 ± 0.19 ($C_v = 6.1\%$) and 20.2 ± 0.21 ($C_v = 6.2\%$), respectively.

Incubation at 10% CO_2 in the first 6 days led to a reduction in the length of the forewings and hindwings, on average, by 16 and 15.1%, respectively, and the number of frenula on them decreased by 3.2%. In the bees that developed under these conditions in the last 6 days, the length of the left forewing and hindwing was reduced by 4.8 and 13.7%, respectively, and the number of frenula 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 $5 \pm 1\%$ CO_2 during the entire period from the prepupal to adult stage, the length of the proboscis and the left forewing and hindwing decreased by 6, 6.7, and 5.4%, respectively, and the number of frenula on them decreased by 1.5%.

Under the influence of elevated 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. In the bees that developed at 15% CO_2 concentration, the right wings became longer than the left ones.

The morphological abnormalities expressed in underdeveloped wings and proboscis were only in those bees that developed in a gas medium with the CO_2 content greater than 5%. Wings were absent (there were only their anlages or folded wing plates) in 5.1 ± 1.4 and $14 \pm 2.4\%$ of bees developing at 10 and 15% CO_2 concentration, respectively. These bees had an underdeveloped proboscis. The minimum length of the proboscis in the former and latter was 4.5 and 4.2 mm, respectively, and the mean values were 5.47 ± 0.327 and 4.65 ± 0.401 mm, respectively.

Hypoxia (10–12% CO_2 concentration) in the initial and final stages of development of the sealed brood had different teratogenic effects. The development under these conditions in the first 6 days after sealing the honeycomb cells led to underdevelopment of wings and proboscis in $33 \pm 11.7\%$ of bees. The number of such bees decreased by a factor of 2.6 ($P \geq 0.99$) if the bees were exposed to hypoxia in the last 6 days of development in the sealed honeycomb cells.

Hypothermia under hypoxic conditions. The effect of hypoxia on the viability and development of bees was traced in the sealed brood developed in the hives of families until the end of the larval stage and unsealing 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, broods were incubated under a natural air environment at 34°C. Broods that were not subjected to cooling were simultaneously incubated with free access of air (Es'kov et al., 2013b).

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 $15.1 \pm 3.1\%$ of bees at the end of development under optimal conditions. Of the brood that was subjected to cooling for 3 h, $20.4 \pm 7.6\%$ 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 elimination of $26.6 \pm 4.9\%$ 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 and 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 loss was 0.4 ± 0.2 and $40 \pm 5.2\%$, respectively.

Cooling and hypoxia of 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 bees incubated under optimal conditions was 12.43 ± 0.21 ($C_v = 9.3\%$), 37.95 ± 0.58 ($C_v = 8.2\%$), and 57.55 ± 1.81 mg ($C_v = 17.1\%$), 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 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.

The cooling of 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 hindwings decreased by 3.3 and 2.3%, respectively ($P \geq 0.99$). The incubation at 25°C for 20 h led to shortening of the left and right forewings by 3 and 2.2%, respectively, and the left and right hindwings 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, it was by 5.1, 3.7, 5.4, and 4.5%, respectively ($P > 0.99$). The reduction in the length of hindwings was accompanied by changes in the number of frenula on them.

Cooling and hypoxia of 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 at 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 ($P > 0.999$).

RESPONSE OF ADULT BEES TO OXYGEN STARVATION

The CO_2 content in the 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.

Ethological and physiological effects of intrahive variability in the CO_2 concentration. Elevated CO_2 levels in the hive space stimulate bees to aerate the nest by flapping wings. The number of bees flapping wings (ventilator 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 ventilator bees is stimulated by an increase in the CO_2 concentration in the periphery of the hive by 1 and 3%, respectively. Ventilator bees respond to an increase in the CO_2 content by increasing the frequency of wing flaps (Es'kov, 1992).

The subthreshold CO_2 concentrations, which do not stimulate the aerating activity, may have an inhibitory effect on bees, which was shown for bee colonies wintering under temperature-controlled conditions (Es'kov, 1995). In the families 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 the 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 ($r = 0.1 \pm 0.21$). The CO_2 concentration in the above-nest space of different families 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 families 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 in the bees that wintered at 7 ± 2°C, the correlation coefficient was -0.62 ± 0.19. This difference was apparently due to the fact that an increase in temperature enhances the effect of hypoxia, consisting in the reduction of the potential possibility of 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 correlation coefficient between the CO_2 concentration in the above-hive space and the content of nitrogen and fat in the body of bees at the end of the wintering period was -0.58 ± 0.17 and -0.48 ± 0.16 (Es'kov, 1995, 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 total or partial immobilization of bees. Bees are anesthetized when the content of CO_2 in the gas medium 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 30% CO_2 atmosphere (Deyme and Belgue-Deyme, 1977) and within 15–30 s in 100% CO_2 atmosphere (Es'kov, 1995).

The time required to activate the anesthetized bees, other things being equal, depends on the duration of anesthesia and the age of bees (Es'kov et al., 2013b).

After incubation in 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 temperature. The effect of hypoxia at a temperature promoting the cold torpor 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 elimination of approximately 50% of bees for 4.4 ± 0.4 h; at 35°C, for 1.7 ± 0.2 h; and at 45°C, for 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 cold torpor 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 in the worker bees strongly depends on their age (Es'kov, 1995). 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 time 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 (Es'kov et al., 2013a).

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 and Jaycox, 1974; Rindfleisch, 1977; Tustain and Faulke, 1979).

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

Anesthesia with CO_2 caused 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 anaesthetized bees decreased, on average, by 11.5, 6, and 6.1%; after incubation at 35°C, these parameters decreased by 13.5, 7.4, and 7.6%, respectively ($P > 0.99$). Incubation for the same time under free access of air caused no significant changes in the weight of the body and its regions.

When bees stayed in the chill-coma state in the natural air at 0°C for 3 h, the weight of 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.

The body weight dynamics under the influence of hypoxia depended on the age of 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, at 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 the 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. In the torpid bees in a natural air environment and 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.

Hypoxia of bees affects the age dynamics of the development of exocrine glands. In particular, 20-min anesthesia of 1- to 3-day-old bees led to the underdevelopment (difference in size) of the hypopharyngeal glands at an age of 14–21 days by an average of 45 ± 2%. The development of the wax glands and the fat body associated with the functioning of these glands was also delayed. The height of the wax glands was 35 ± 5 μm (maximum, 62 μm) versus 50–60 μm (maximum, 80 μm) in the unanesthetized bees, and the fat body hypoplasia reached 6–30% (Skowronek and Jaycox, 1974).

Age-related variation in polyethism in worker bees. Functional differentiation of working bees in a bee family is not related to their external differences but depends on the age (Langstroth, 1909; Phillips, 1930). Anesthesia disturbed the age sequence of polyethism in bees. 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 and 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 and Jaycox, 1974). After anesthesia at an age of 10 days, the flight activity of forager bees first increased and then (in approximately 5 days) sharply decreased and became even lower than that of the unanesthetized bees. Anesthesia of 20-day-old bees did not stimulate their flight activity. Conversely, it drastically decreased 2–3 days after anesthesia. Since that time, all bees completely ceased to deliver pollen, whereas approximately one-third of unanesthetized bees of the same age returned to the hive with pollen.

Development and attractiveness of queens. Anesthesia of queens at the beginning of the imaginal stage accelerates their development. They start ovipositing earlier than the 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 approximately 1.5 times the number of their mating flights, which began and ended later than the flights of the unanesthetized queens. After the completion of mating flights, the sperm count in the spermathecae 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 anesthesia decreased 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 spermathecae 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, anesthesia of queens reduced their lifespan (Skowronek, 1979). Apparently, this was due to the intensification of the physiological aging of queens.

CONCLUSIONS

A bee brood can normally develop only in a narrow temperature range with an optimum at 33–34.5°C. Deviations beyond the limits of this range by 3 and 5°C determine average and absolute, respectively, lethal efficiency. Conversely, an excess in the CO_2 concentration by two orders of magnitude relative to the CO_2 content in the hive at an optimal temperature for bee families 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 up to 10–15% cause a significant decrease in the viability of bees, shortening the proboscis, and partial or complete underdevelopment of wings. The shortening of wings is accompanied by a change in the symmetry because the right wing becomes longer than the left one.

The development of bees at elevated CO_2 concentrations and/or low temperature leads to an increase in the body weight at the adult stage. Such bees are also characterized by a high content of water in the cephalic, thoracic, and abdominal regions of the body, which is indicative of a water metabolism disorder in them. Hypoxia has the opposite effect on adult bees. In the latter, anesthesia in with CO_2 drastically reduces

the body weight, which is mostly due to water losses. This process is potentiated by a temperature increase and inhibited by a temperature decrease to the chill-coma state. Since in the chill-coma state and anesthesia the respiratory function is inhibited, water transport occurs primarily through the body integuments.

In many species of solitary living insects, the protection from hypoxia and many other adverse effects of the environment is ensured in the state of diapause. For the honeybee, diapause is biologically inappropriate, because the feed stocks of the family and bees themselves need active protection from predators and robbers. For this reason, during the adaptation of the honeybee to temperate and cold climate, natural selection has favored the development of an effective mechanism of thermoregulation and high tolerance to hypoxia. In the combination of these adaptations, the honeybee is distinguished and unparalleled in the animal kingdom.

REFERENCES

Austin, G.H., Effect of carbon dioxide anesthesia on bee behavior and expectation of life, *Bee World*, 1955, vol. 36, no. 3, pp. 45–47, no. 4, pp. 62–72, no. 5, pp. 81–92, no. 6, pp. 105–111.

Budel, A., Schwankungen der lufttemperatur in der wabengasse eines brutenden bienenvolkes, *Z. Bienenforsch*, 1955, no. 3, pp. 88–92.

Deyme, A. and Belgue-Deyme, G.J., Determination de la dose narcotique de gaz carbonique (CO_2) en junction de l'ouvrienre d`abeille (*Apis mellifera* L.), *Apidologie*, 1977, vol. 8, no. 3, pp. 217–228.

Es'kov, K.K. and Toroptsev, A.I., Microclimate of bee nest as a factor influencing the development of queens, *Zh. Obshch. Biol.*, 1978, vol. 39, no. 2, pp. 262–275.

Es'kov, E.K., *Etologiya medonosnoi pchely* (Honeybee Ethology), Moscow: Kolos, 1992.

Es'kov, E.K., *Ekologiya medonosnoi pchely* (Ecology), Ryazan': Rus. Slovo, 1995.

Es'kov, E.K., Individual and social adaptation of the honeybee to wintering, *Usp. Sovrem. Biol.*, 2003, vol. 123, no. 4, pp. 383–390.

Es'kov, E.K. and Es'kova, M.D., The physiological effects of hypoxia on developing and adult bees *Apis mellifera* L., *Zh. Evol. Biokhim. Fiziol.*, 2011, vol. 47, no. 6, pp. 469–474.

Es'kov, E.K., Es'kova, M.D., and Spasik, S.E., Changes in body weight and lifespan of bees *Apis mellifera* L. under the influence of anesthesia with carbon dioxide, *Zh. Evol. Biokhim. Fiziol.*, 2013a, vol. 49, no. 49, pp. 459–461.

Es'kov, E.K., Es'kova, M.D., and Spasik, S.E., Development of bees from prepupal to adult stage under hypoxia, *Vestn. Ros. Akad. S-Kh. Nauk*, 2013b, no. 4, pp. 57–58.

Fillips, E.F., *Pchelovodstvo* (Beekeeping), Moscow: Sel'khozgiz, 1930.

Heinrech, B., The social physiology of temperature regulation in honeybees, *Forschr. Zool.*, 1985, vol. 31, pp. 393–406.

Hess, W.R., Die temperaturregulation im bienenvolk, *Zeit. Vergl. Physiol.*, 1926, no. 4, pp. 465–487.

Langstrot, L.L., *Pchela i ulei* (Bee and hive), St. Petersburg: Izd. A.F. Devriena, 1909.

Mackensen, O.W., Effect of carbon dioxide on initial ovipositor of artificially inseminated and virgin queen bees, *J. Econ. Etomol.*, 1947, vol. 40, pp. 344–349.

Michener, C.D., *The Social Behaviour of the Bees*, Cambridge: Massachusetts. Harvard Univ. Press, 1974.

Moeller, F.E., Development of hybrid bees, *Prod. Res. Rept NS Dep. Agricultural.*, 1976, no. 168, pp. 1–11.

Rindfleisch, J.K., The use of nitrous oxide as an anesthetic for honey bees, *Amer. Bee J.*, 1977, vol. 117, no. 10, p. 622.

Seeley, T.D., Atmospheric carbon dioxide regulation in honey-bee (*Apis mellifera*), *J. Insect Physiol.*, 1974, vol. 20, pp. 2301–2305.

Skowronek, W. and Jaycox, E.K., Wplyw dwutienku wegla na pzczoly robotnice, *Pszel. Zesz. Nauk. Pulawy*, 1974, vol. 18, pp. 107–119.

Skowronek, W., Biologia unasceniania sie matek pszezelich usypianych dwutlenkien wegla, *Pszczel. Zesz. Nauk. Pulawy*, 1976, vol. 20, pp. 99–115.

Skowronek, W., Wplyw dwutlenku wegla na wartose uzytkowa matek pszczelich, *Pszezel. Zesz. Nauk. Odd. Pulawach. Inst. Sad. I Kwaiaciar*, 1979, vol. 23, pp. 89–96.

Tustain, R.C.R. and Faulke, J., Effect of carbon dioxide an aesthesia on the longevity of honey bees in the laboratory, *N. Z. J. Exp. Agr.*, 1979, vol. 7, no. 3, pp. 327–329.

Translated by M. Batrukova