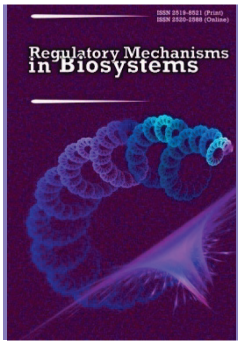

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Ontogenesis of honey bees (*Apis mellifera*) under the influence of temperature stress

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The productivity of bee colonies mostly depends on the influence of exogenous factors. The most significant ones include: the presence of a honey base, climatic conditions, environmental temperature, etc. The stability of microclimate indicators in the nest significantly affect the growth of families and nest. The vital temperature range in the post-embryonic period ranges from 30–38 °C. For normal brood development, the incubation temperature should be 34–35 °C. The intensity of growth during this period is largely determined by the influence of climatic conditions, including temperature. As a result of decrease in temperature, there is a densification of the placement of bees, which are more concentrated in the central, breeding part of the nest. Under these conditions, hypothermia of individual areas of the brood, located on the peripheral parts of the honeycombs, beyond the healthy temperature range, is sometimes noted. This is manifested by a sharp drop in the ambient temperature, when there is a numerous brood in the family, or if the nest is not expanded properly. In order to study the influence of cold stress on the development of offspring, two groups were formed. The group incubated at a temperature of 35 ºC was considered the control group. The experimental group was a group which was grown at a temperature of 32 ºC. After the brood was sealed, pupae were selected every 2 days. At the same time, their mass, the content of total lipids and the ratio of their individual classes were determined. It was found that under the influence of cold stress during brood development, the duration of the incubation period increases by 35–42 hours. It has been established that the adaptation of bees to cold includes a complex of physiological and biochemical processes, among which changes in the mass and composition of lipids play a significant role. The dynamics of triacylglycerides under optimal and stressful conditions of incubation were studied. Taking into account their functional purpose as the main element of energy supply of metabolic pathways, such dynamics indicate profound changes in the processes of energy exchange. When studying the mechanisms of adaptation to cold, a change in the ratio of phospholipid classes, the expression of which is induced by the effect of cold, was revealed. The results of this study bring a deeper understanding of the adaptation mechanisms of honeybees' response to changes in brood incubation temperature. They expand data on the biology of bees during critical periods of growth and development.

Keywords: honey bees; ontogenesis; brood; cold stress; brood incubation; lipolysis; phospholipids; triacylglycerides.

Introduction

According to the growth and development of larvae, complex, intensive metabolic processes take place in their organisms (Brejcha et al., 2023). In the first phase of development, they are aimed mainly at the accumulation of nutrients, which in the next phase are used to form the body of adult insects and ensure the energy needs for this process. As per (Zoltowska et al., 2011), the bee larva reaches its maximum body weight on the sixth day of the post-embryonic stage of development, before the cell is sealed. During the period of spinning the cocoon, the mass of the larva begins to decrease sharply, which is a physiological process caused by the consumption of reserve nutrients for the formation of organs and body systems. The decrease in body weight continues directly into the imago period, resulting in a total weight loss of about 30% (He et al., 2016). Such dynamics of body weight occurs under the condition of brood incubation in the optimal temperature regime. When consuming food, in the process of vital activity, adults and offspring release the end products of their metabolism: heat, water vapor, carbon dioxide and (in small quantities) other vital products (Kovac et al., 2018). As a result, a certain temperature is formed in the nest, which is one of the components of the microclimate. Simultaneously, the microclimate that is formed has a direct effect on the individuals themselves. Honey bees belong to animals that do not have a constant body temperature. They have developed behavioral thermoregulation, which manifests itself in two forms. In some individuals – in the form of a thermal preference, that is, a directed search for the temperature of the external environment. Bees also have a social effect aimed at maintaining an optimal microclimate. The mechanism of heat generation in the body of honey bees has been studied (Zhao, 2021). The release of heat is the result of micro vibrations of the pectoral muscles of bees (Stabentheiner et al., 2010). Adaptation of animals to cold includes a complex of biochemical processes, including changes in the content of water, carbohydrates, and the composition of lipids and proteins (Mucci et al., 2021). At the same time, the question of changes in lipid metabolism in response to a decrease in the temperature of the environment which honey bee broods inhabit is still understudied. It is known that a sharp decrease in the temperature of the external environment or improper expansion of the bee nest leads to cooling of the brood, especially in the peripheral areas. At the same time, it is not known how they will react to cooling in the pre-pupa and pupa phase, and whether the bees will be able to functionally perform the work of raising brood and collecting nectar. Therefore, the purpose of the article was to study the nature of some physiological and biochemical changes in the body of the honey bee at different stages of ontogenesis under the influence of temperature stress.

Materials and methods

The research was conducted in the laboratory on the basis of the educational and production apiary of the Department of Small Animal Production and Processing Technology of the S. Z. Gzhitskyi Lviv National University of Veterinary Medicine and Biotechnology, in particular on bees of the Carpathian breed of the interbreed type "Synevyr".

In accordance with European legislation (EU Directive 2010/63/EU8), no special permits are required for the use of honey bees for this study. Four bee families were selected for the research using the method of analogues. All queens were obtained from one maternal family and one inoculation of queen rearing larvae. To obtain a one-year-old brood, in the first decade of May, queens were placed in frame insulators on one light-brown honeycomb previously prepared by bees for egg-laying. After laying the first eggs, the queens were kept in the isolators for the next 10 hours, after which they were released, and the experimental cells, without the isolators, were moved to the nest of the same family. In this way, the same conditions for raising offspring were ensured. In order to prevent further laying of eggs by the queen in experimental combs, they were separated from the breeding part of the nest with a separate grid, which made it possible to avoid the appearance of broods of a different age in these combs. Breeding was carried out in a family that had about 9 kg of carbohydrate feed. A sugar syrup solution was used as a source of carbohydrates. Lipid-protein nutrition was provided by placing 0.9 kg of perga in the nest. After sealing all the cells with the brood (on the 6th day of the larval stage), all the experimental cells were divided vertically into two equal parts and continued to grow the brood in incubators. At the same time, one of the parts of each cell, which was included in the control group, was grown at a normal stable temperature of 35 °C, and the other (experimental group) was grown in a stressful regime, under conditions of reduced temperature (32 °C), which corresponds to the level of the minimum healthy range brood rearing temperature (Ramirez et al., 2020). The relative humidity of the air was kept at the level of 80%. Lyson thermostats were used to incubate the brood, which ensures the accuracy of maintaining the microclimate parameters at a temperature of up to 0.1 °C and a relative humidity of up to 1%. In order to study the influence of brood incubation temperature, namely its closed phase of development, on physiological, biochemical and morphological indicators, starting from the pre-pupa stage, 20 pupae were selected from each part of the investigated combs 6 times with a frequency of 48–72 hours. At the same time, dry weight, content of total lipids, ratio of classes of lipids, phospholipids, and esterified cholesterol were determined. The raw body weight of the pupae was determined by weighing on analytical scales, and the dry weight was determined after drying in an oven at 102 ºC, monitoring the change in the initial weight of the samples every 24 hours. Samples were dried to a constant final weight. The water content in their bodies was determined by the difference between the wet and dry mass of pre-pupae and pupae. The content of total lipids in the dried material was determined according to the method Folch (1957), the ratio of phospholipid classes according to the method Brockhuyse (1969). The results of biochemical studies are given according to the International System of Units, recommended for use in clinical laboratory practice.

One-way analysis of variance (ANOVA) was used to identify significant differences between the studied groups. In the case of using absolute indicators, $P < 0.05$ was considered a statistically significant difference.

Results

At the first stage of the research, the age dynamics of the body weight of individuals was determined in the conditions of brood development under a stable temperature regime. At the beginning of the experiment (zero count), the body weight of the larvae after sealing in the experimental groups was almost the same and amounted to 156.48 ± 0.86 mg in the control and 156.53 ± 1.11 mg in the experiment. In the period from the 9th day of development, including the imaginal stage, which in the control occurred on the 21st day after the laying of the egg by the uterus, the body weight of individuals gradually decreased (Table 1).

Table 1
The effect of reduced incubation temperature of closed brood in the healthy range of the body weight of working individuals (x ± SD, n = 8)

The ontogenesis period of the closed brood phase	The stage of brood development		The body weight, mg		The difference between control and experiment, %
	control	experiment	control	experiment	
Capping of the cells (neutral countdown)	larva	larva	156.48 ± 0.86	156.53 ± 1.11	–
2 day	prepupae	prepupae	154.84 ± 1.59	154.95 ± 0.66	0.07 ± 0.02
4 day	pupae	pupae	149.13 ± 0.24	154.33 ± 0.80	3.49 ± 0.06**
6 day	pupae	pupae	145.96 ± 0.27	153.21 ± 0.31	4.97 ± 0.06***
8 day	pupae	pupae	142.10 ± 0.14	152.89 ± 0.76	7.59 ± 0.73***
11 day	imago	pupae	131.04 ± 0.69	149.47 ± 0.25	14.06 ± 1.48***
13 day	–	imago	–	139.13 ± 1.23	6.17 ± 1.15***

Note: probable differences in the indicators of the research group compared to the control group (* – P < 0.05, ** – P < 0.01, *** – P < 0.001); the number of parts of combs with broods involved in the conducted research; at each sampling, 20 individuals from each part of the comb are used.

However, the dynamics of weight loss in the experimental group was significantly slowed down, and this trend was observed throughout the closed breeding phase and had a more dynamic nature. As a result, as the body weight decreased during development, the difference in the weight of the same-aged individuals in the control and experiment steadily and reliably increased until the 11th day of the development of the closed brood phase (from 0.11 mg on the 2nd day of the pre-pupa to 18.43 mg on the 11th day, $P < 0.001$). At the same time, changes in the duration of development in the closed brood phase were detected. Lowering the incubation temperature to 32 °C at this stage caused a time extension until the emergence of the imago of the experimental group within 35–42 hours. However, regardless of this delay in development, adults at the time of leaving the cells differed by a larger body weight, compared to the control group, on average by 8.09 mg ($P < 0.001$). In percentage terms, the difference in mass was 6.17%.

In the process of studying the dynamics of body weight, it was established that the raw weight of pupae during the closed brood phase in the control group decreased by 16.26%, and in the experimental group, at a reduced incubation temperature, by 11.12% (Table 2).

A factor in the slow dynamics of body weight reduction in the conditions of the conducted research was the reduced brood incubation temperature. In this situation, the question of the biological mechanism that caused such inhibition of mass dynamics and temporal prolongation of the brood incubation period remained unexplained. In this context, methodologically, first of all, the dynamics of dry matter mass and water content should be divided and studied separately in experimental groups of families (Table 3). As is observable from the data in Table 3, during the entire phase of closed breeding in both groups, equally negative dynamics of dry body weight of individuals were recorded. At the same time, in the experimental group, it showed a slight but reliable slowing ($P < 0.001$), as a result of which, after sealing the larvae and up to the imago stage, the dry weight of the body in the control group decreased by 49.8%, and in the experiment – by 41.8%.

Table 2
The dynamics of age-related changes in body weight of individuals during the closed brood phase depending on the incubation temperature (x ± SD, n = 8)

The ontogenesis period of the closed brood phase	Change in initial body weight of individuals, %	
	control	experiment
2 day	-1.05 ± 0.03	-1.01 ± 0.06
4 day	-4.70 ± 0.06	-1.41 ± 0.03**
6 day	-6.72 ± 0.03	-2.12 ± 0.04***
8 day	-9.19 ± 0.02	-2.33 ± 0.03***
11 day	-16.26 ± 0.08	-4.51 ± 0.01***
13 day	imago	-11.12 ± 0.03***

Note: see Table 1.

Table 3
The effect of the prior vital of temperatures on the dry weight and water content in the body tissues of workers (mg, x ± SD, n = 8)

The ontogenesis period of the closed brood phase	Dry substance		Water	
	control	experiment	control	experiment
Capping of the cells (neutral countdown)	35.47 ± 0.46	35.43 ± 0.93	121.01 ± 0.16	121.10 ± 0.91
2 day	34.63 ± 0.25	33.92 ± 0.26	120.21 ± 0.35	121.03 ± 0.90
4 day	31.30 ± 0.54	33.52 ± 0.05**	117.83 ± 0.18	120.81 ± 0.54**
6 day	29.66 ± 0.16	30.64 ± 0.06***	116.30 ± 0.94	122.57 ± 0.40***
8 day	26.49 ± 0.02	29.33 ± 0.22***	115.61 ± 0.76	123.56 ± 0.20***
11 day	17.79 ± 0.12	23.06 ± 0.80***	113.25 ± 0.75	126.41 ± 0.26***
13 day	Imago	20.62 ± 0.36***	imago	118.51 ± 0.02***

Note: see Table 1.

This was reflected in the dry weight of adults immediately after leaving the cell, which was on average 2.83 mg more in the experiment, which is a percentage of 15.9% (P < 0.001). During the pupal stage, stable dynamics of tissue dehydration were recorded in the control group. The water content decreased by 6.41% – from 121.01 mg (after the brood was sealed) to 113.25 mg (when the imago emerged). Variable dynamics of tissue hydration were found in the research group. From the beginning of cell sealing to the 4th day of development, a slight decrease in water content was noted, after which a positive dynamics of tissue hydration was established until the 11th day, as a result of which the water content increased. In the period from 11am to 1pm, a sharp decrease in water content was recorded. As a result of such dynamics of hydration, during the period of the closed brood stage in the experimental group, the water content decreased by 2.1% – from 121.10 mg (after the brood was sealed) to 118.51 mg (when the imago emerged). An individual of the imaginal stage of the experimental group, immediately after hatching, contained an average of 5.26 mg more water than in the control (P < 0.001). In percentage terms, this difference is 4.6%. The purpose of the next stage was to study the influence of the incubation temperature regime on the dynamics of the content of total lipids in the body of honey bees and the ratio of their classes during the development of the closed brood phase. The maximum content of total lipids in the body of individuals (23.16 ± 0.46 mg in the control and 23.11 ± 0.55 mg in the experiment) was recorded at the larval stage during the sealing period. In the process of metamorphosis of the larva into the adult stage, the content of total lipids in the control decreased by 77.2% (to 5.28 ± 0.50 mg – when the adult emerged). In the experiment, during the development phase of the closed brood, the lipid content decreased by 66.7%. When the bees left the cells, the lipid mass was on average 7.69 ± 0.91 mg per individual, which is 45.6% more than in the control (P < 0.001). A higher content of total lipids in individuals of the experimental group was recorded throughout the incubation period (Table 4).

The general lipids are represented by several classes: phospholipids, mono- and diglycerides, free cholesterol, non-esterified fatty acids, triacylglycerides, esterified cholesterol. Triacylglycerides play a leading role in energy exchange, and the structural function is mainly performed by phospholipids, which are part of the protein-lipid bilayer of cell membranes.

The dynamics and ratio of these classes of lipids during the development of the closed phase of the brood under different temperature conditions of incubation are shown in Table 5. The age-related dynamics of the percentage content of phospholipids and triacylglycerols (in the ratio of lipid classes) in both groups has revealed a variable character. Although, in the control, in each period of ontogenesis, a higher content of phospholipids was found. In addition, the content of phospholipids prevailed over the content of all other classes of lipids.

Table 4
The effect of vital range of brood rearing temperatures on the content of total lipids in the body of individuals (x ± SD, n = 8)

The ontogenesis period	Mass of the total lipids, mg/individual		The content of total lipids in terms of dry substance, %	
	control	experiment	control	experiment
Capping of the cells (zerol countdown)	23.16 ± 0.46	23.11 ± 0.55	65.29 ± 0.15	65.23 ± 0.05
2 day	22.23 ± 0.64	22.03 ± 0.17	64.19 ± 0.60	64.95 ± 0.08
4 day	17.28 ± 0.81	19.41 ± 0.49***	55.21 ± 0.18	57.91 ± 0.06***
6 day	15.53 ± 0.46	17.14 ± 0.84***	52.25 ± 0.67	55.94 ± 0.01***
8 day	10.90 ± 0.65	14.86 ± 0.86***	41.15 ± 0.66	50.66 ± 0.03***
11 day	5.28 ± 0.50	9.34 ± 0.80***	29.68 ± 0.32	40.50 ± 0.05***
13 day	imago	7.69 ± 0.91***	–	37.29 ± 0.08***

Note: see Table 1.

Table 5
The age dynamics and the ratio of phospholipids and triacylglycerols in the individuals depending on the brood incubation temperature (%, x ± SD, n = 8)

The ontogenesis period	Phospholipids		Triacylglycerols	
	control	experiment	control	experiment
Capping of the cells (zerol countdown)	28.56 ± 0.16	28.42 ± 0.64	24.84 ± 0.45	24.46 ± 0.25
2 day	27.74 ± 0.74	24.97 ± 0.75*	20.75 ± 0.97	30.91 ± 0.87***
4 day	30.74 ± 0.57	24.52 ± 1.81*	26.28 ± 0.53	26.06 ± 0.21
6 day	30.58 ± 0.90	21.89 ± 0.60***	30.23 ± 0.79	23.08 ± 0.61**
8 day	28.05 ± 0.40	21.82 ± 0.76**	17.07 ± 0.79	26.41 ± 0.46*
11 day	31.15 ± 0.51	23.03 ± 0.32***	21.82 ± 0.76	24.71 ± 0.24**
13 day	–	27.44 ± 0.18	–	21.14 ± 0.74

Note: see Table 1.

In the research group, up to the 11th day of development of the closed brood phase, a higher content of triacylglycerides was found. On the last, 13th day of brood development, the ratio of these lipid classes shifts towards phospholipids (similarly to the control group).

Discussion

One of the main factors that has a decisive influence on the life and development of honey bees is the optimal temperature regime of the nest in the breeding area, which varies between 34–35 °C (Zhao, 2021). This is due to the fact that honey bees evolved in tropical climates and adopted the strategy of raising broods at relatively high temperatures. With a decrease in temperature, an increase in the number of deaths of offspring was recorded. High larval mortality is noted at low temperatures in the range of 5–24 °C, especially after 72-hour exposure (Ramirez et al., 2020). At the same time, there is data that honey bees, in particular drones that were incubated at a temperature of 32 ºC, undergo changes associated with an increase in body weight (Szekońska & Tofilski, 2021). However, the authors do not reveal the physiological reasons for this phenomenon. Some researchers believe that bee health depends on body mass, indicating that the greater the mass, the better the physiological indicators (Bowen-Walker & Gunn, 2001, Regatschnig et al., 2014, Regatschnig et al., 2021). Our research reveals the regulatory mechanisms of such causes during the incubation period of bees. And in some cases, existing theories about the mass of bees in ontogeny are refuted.

The collective behavior of the workers of the bee colony as a complete biological unit is aimed at maintaining the temperature necessary for brood growth (Kodrík et al., 2022). The reduced temperature regime for bees in the process of evolution contributed to the emergence of a number of adaptive protective reactions in them. In the process of maintaining the optimal temperature, individual members of the family participate, which ensures effective heat transfer and accurate thermoregulation (Kleinhenz et al., 2002). At the individual level, heat generation is the result of micro vibrations of the thoracic muscles of individual workers (Stabentheiner et al., 2010).

A decrease in the ambient temperature or technological errors made, in particular, during the expansion of the nest of the bee colony, can lead to the stay of part of the brood in areas with suboptimal microclimate parameters. This especially applies to the range of low temperatures, which for the honey bee (*Apis mellifera*) varies between 30–32 °C (Ramirez et al., 2020). The consequences of brood incubation in the vital range of low temperatures are manifested in the individual characteristics of adult workers grown from this brood, in the form of morphological disorders in the development of various systems and organs, their physiological activity, behavioral reactions, shortening of lifespan, etc. (Ken et al., 2005; Jones et al., 2005; Rueppell et al., 2007; Tautz et al., 2009). A decrease in the incubation temperature of bee broods led to a decrease in the length of the proboscis and abdomen of adult bees, as well as an increase in the length and width of the forewing (Becher et al., 2009). At the same time, adults may suffer from nervous and behavioral insufficiency.

The duration of development at the pupal stage also depends on the brood incubation temperature regime. A decrease in temperature slows down development, increasing its duration, and an increase accelerates it, shortening its duration. Slowing down of development with a decrease in temperature is due to inhibition of metabolic processes, which was experimentally confirmed by the intensity of oxygen consumption by larvae. A reduction in the workload of honey bees, a decrease in the intensity and duration of mobilization dances, and a decrease in the flight activity of these bees negatively affect the overall productivity of bee colonies, as these consequences arose as a result of cold stress (Ramirez et al., 2020).

Such phenomena became especially acute with the development of modern agricultural technologies, when bees faced new challenges of abiotic and biotic origin. The use of various classes of pesticides, the mass unsystematic use of antibiotics, and the deterioration of the ecological situation of the environment have recently led to a significant decrease in the immunity of bees (McMullan et al., 2005). A number of researchers indicate a greater vulnerability to pesticide poisoning and a shorter life span of bees exposed to reduced temperatures in the healthy range during the brood stage (Medrzycki et al., 2010).

A number of authors investigated the influence of brood incubation temperature on the reproductive value of honey bees (*A. mellifera*). Drones incubated at 32 °C had larger reproductive organs and a higher percentage of live sperm. On the other hand, drones incubated at 35 °C were more likely to have higher sperm counts (Czekońska et al., 2015). The cold-stressed broodstock quickly resumes growth in the first 24 hours after returning to the control rearing temperature, which is supported by the induction of compensatory mechanisms (Ramirez et al., 2020). The results of a number of studies have shown that a lower brood rearing temperature had no significant effect on the mortality of larvae, but strongly influenced the mortality of adult bees. Moreover, adult bees that emerged from cells at a temperature of 33 °C were significantly more susceptible to the pesticide dimethoate. However, larval LD_{50} (48 h) was 28 times higher at 33 °C than at 35 °C. The authors explain the significant differences between larvae and adults by the different metabolism of the larvae at 33 °C and, as a result, the slow absorption of the active ingredient. Therefore, adult honey bees raised at suboptimal brood temperature may be more vulnerable to pesticide poisoning and have a shorter lifespan (Medrzycki et al., 2010). A large number of authors found that longer low temperatures during the closed brood phase resulted in higher mortality. A greater number of cases of disorientation of larvae inside the cells and a shorter lifespan of worker bees were recorded. Pupae were more sensitive to exposure to low temperatures, while larvae showed the highest resistance to low temperature stress. Research findings suggest that the pupal stage is more sensitive to low temperature stress presumably because of the many physiological changes associated with metamorphosis that occur during this period (Wang et al., 2016). Research has also established that growing pupae at various stable temperatures from 29 to 37 °C leads to neuroanatomical changes in the brains of adults (Groh et al., 2004).

The reduced incubation temperature in the closed brood phase affects the development of the muscles of individuals at the imago stage, since even adult workers need a high temperature of 35 °C for the maturation of pectoral muscles (Wang et al., 2016). In addition, the activity of many enzymes, in particular those related to the synthesis of juvenile hormones, can be disturbed under the influence of low temperatures, which leads to the death of pupae and subsequent negative impact on individuals of the imaginal stage. Although after exposure to cold stress, most of them emerged from the brood and had normal morphology, the lifespan of these individuals was significantly reduced. Accordingly, there is no understanding of the mechanisms by which adults grown at low temperatures look morphologically complete, and their life span is shortened. Particularly, Chinese researchers point to the lack of understanding of the main biological mechanisms that determine the reduced lifespan of adult workers with a visually normal morphology that were exposed to cold stress at various stages of the closed brood (Wang et al., 2016).

As stated by data (Zhao, 2021), the influence of strong deviations from the normal brood temperature leads to increased mortality and morphological deficiency. Several authors noted that bees reared at 32 °C completed only about 20% of the dance circles compared to bees in the higher temperature group (Tautz et al., 2003). Several researchers have studied the mechanisms of social thermoregulation of bee families when the environmental temperature changes (Ramirez et al., 2017). An increase in cold stress leads to an increase in heat production by the pectoral muscles. Endothermic heat production is the work of bees older than two days. All of them are equally engaged in active heat generation, both in terms of intensity and frequency. Their active heat generation has an important effect of increasing metabolic processes to accelerate the development of pectoral muscles (Stabentheiner et al., 2010).

In the presence of a brood, more accurate thermoregulation is observed in bees (Khoury et al., 2013). Bees that heat up can be distinguished from those that are resting not only by a higher body temperature, but also by the continuous, rapid breathing movements of their abdomens. In contrast, in resting bees, abdominal pumping movements are intermittent and punctuated by long pauses (Kleinhenz et al., 2003).

Bee colonies (*A. mellifera*) maintain a temperature of 34–35 °C in the brood nest, as high and stable temperature conditions are required for optimal brood development. It has been shown that brood incubation at the level of individual bees is carried out by workers who perform a certain and yet to be determined behavior: they raise the temperature of the brood by firmly pressing their warm breasts to the lids of the cells under which the pupae develop (Johnson, 2010). Bees remain immobile in a characteristic posture and have a significantly higher breast temperature than bees that do not occupy this posture in the brood zone. The surface of the lids of brood cells, to which warm bees pressed their thorax, was 3.2 °C higher than the surrounding area. This is confirmation that effective heat transfer has occurred (Bujok et al., 2002).

In spite of the conducted studies concerning the negative impact of cold stress of bee brood on the morphological parameters and biological indicators of workers (Czekońska et al., 2013), the question of the mechanism of the cold factor's influence at the physiological level remains unclear. In this context, it is of great interest to find out the effect of low temperatures on the physiological and biochemical processes in the body of pupae.

The answers to these questions consist in the peculiarities of brood incubation processes. It is known that the supply of nutrients is deposited in the pool of metabolites of the fat body of the larva, which reaches its maximum level of development on the 9th day (Brejcha et al., 2023). At the end of this period, the larva stops feeding. One part of the metabolite fund begins to be spent for the renewal of the structural and functional components of cells, the formation of systems and organs (anabolism). The second part is broken down (catabolism) into the final products of metabolism, providing the processes of anabolism and functional activity (substance transport, muscle work, heat production, etc.) with the necessary energy (Chen et al., 2022). In accord with the obtained results of the studies, the intensity of the processes of decomposition and synthesis depends on the temperature of brood incubation. Under the optimal temperature regime of brood incubation, the main part of the energy obtained in catabolism in the form of ATP is spent on biosynthesis processes during molting and the formation of new organs and systems. The end products of energy metabolism (in the form of H_2O and CO_2) are removed from the body, and the body weight of individuals, in the absence of external nutrition, gradually decreases. Due to a decrease in brood incubation temperature, metabolic processes are inhibited, and the energy obtained in the process of catabolism is more directed to the processes of heat production, transport of substances, etc., and to a lesser extent to the processes of anabolism. This is evidenced by the slowing down of the dynamics of weight loss in individuals, which is caused by the inhibition of consumption of reserve nutrients from the body's metabolite fund. As a result, the imago stage emerges from the cells with more (excess) mass, which is distributed between dry matter and water.

Adaptation of insects to cold includes a complex of biochemical processes. A key and, at the same time, understudied factor in such an adaptation process are changes in lipid metabolism in response to a decrease in the temperature of the environment. This is due to the fact that lipids, as well as the products of their metabolism, form a whole group of biologically active compounds that have a direct effect on metabolic processes. Energy provision of metabolic reactions, including lipid metabolism, in the closed brood phase (when there is no external nutrition of the organism) occurs due to the breakdown of energy substrates deposited in the body at the larval stage of development. Lipolysis processes, in particular triacylglycerides, as one of the main energy components, occur under the influence of enzyme complexes of lipases, which are also catalysts of histolysis processes of tissues and organs during molting. According to their protein nature, these enzymes are thermosensitive and thermolabile compounds. The increase in the rate of the enzymatic reaction under the influence of the temperature factor occurs in accordance with the Arrhenius equation ($k = Ae - \Delta E/RT$ – the rate constant of the chemical reaction) in the temperature range of 37...40 °C. With a decrease in temperature (to 32 °C in the conditions of conducting research), there is a decrease in the activity of lipase enzymes, as a result of which the rate of lipolysis reactions decreases sharply (Raymond, 2005). This is partly due to the slowing down of the age-related dynamics of the decrease in total body weight under the influence of the temperature factor, when lipids are not fully used to build body tissues.

If the brood incubation temperature decreases, the oxidation energy of energy components, in particular triacylglycerides, is used more for heat production and less for ensuring the course of anabolism reactions for the formation of structural and functional components of the body. The mechanism of this process can partly be caused by free, non-phosphorylated oxidation of substrates, in which the process of tissue respiration on mitochondrial membranes is disconnected from the process of phosphorylation. Coincidentally, the process of splitting lipids in many cases occurs under the influence of hormone-sensitive enzymes. Lipids form the basis of fat body trophocytes, and more than 90% of them are in the form of triglycerides, which are synthesized from dietary carbohydrates, fatty acids, or proteins (Arrese & Soulages, 2010). Hydrolysis of triacylglycerides is carried out with the participation of triacylglycerol lipase, which is in an inactive form and is activated by cyclic adenosine monophosphate, which is also a heat-sensitive compound. Changes in the dynamics of lipid classes and their ratio noted in the course of research require further study and justification.

Conclusion

As a consequence of lowering the incubation temperature of the brood, in particular its closed phase, to the lower limit of the healthy range, the duration of the molting process increases and, accordingly, the general period of development of the brood to the imago stage is prolonged, with a further negative impact on the physiological state of the adult workers of the family. Under the influence of cold stress, the delay in the development of offspring is manifested by the prolongation of the ontogenesis process by 35–42 hours.

In the midst of development of individuals in the closed brood phase, there is a stable decrease in body weight, which is naturally due to the intensive use of nutrients accumulated in the larval stage and the lack of external nutrition. Violation of the optimal parameters of the microclimate during the brood incubation period affects the decrease in the dynamics of the body weight of individuals, as a result of which the body weight of the adults at the exit from the brood at a low incubation temperature was 6.2% higher ($P < 0.001$). Simultaneously, a 4.6% higher water content in the body was found ($P < 0.001$). However, the mass fraction of water in both experimental groups during the entire stage of the closed brood was kept at almost the same level, which indicates the preservation of the water-salt balance of the body, regardless of the change in the temperature regime of growing the brood. Slowing down of the dynamics of mass and dehydration is due to inhibition of metabolic processes, when nutrients deposited in the larva's body, as a result of temperature stress, are less actively and not fully involved in metabolic processes. In consequence, when the temperature conditions of brood incubation are violated, body weight cannot be an assessment marker of the level of physiological development of individuals.

In accordance with the conditions of cold stress, a low intensity of lipolysis was found, as a result of which in the pre-imaginal period the content of total lipids in the body of the individual exceeded the control by 45.6% ($P < 0.001$). Such a change in the dynamics of lipolysis occurs against the background of a slow decrease in the mass of individuals, which is due to a decrease in the intensity of metabolism. Lowered incubation temperature leads to changes in lipid metabolism, which is manifested in the disturbance of the balance of certain classes of lipids, in particular phospholipids and triacylglycerols. In the general run of things, the highest mass fraction of phospholipids was reliably detected during the entire phase of the closed brood at each stage of development. At a low incubation temperature, the ratio of lipid classes until the penultimate day of development is shifted towards an increase in triacylglycerides. Obviously, this factor is one of the factors of pathological deviations in the development of offspring, which manifest themselves at the imago stage in the form of physiological disorders of the body. Along with this, when breeding offspring under stressful low temperatures, a lethal course was found in 8% of cases.

The conducted research has practical application in beekeeping in terms of the importance of ensuring favorable conditions for growing broods, which should include avoiding the fall of broods into the zone of low temperatures, which often occurs in the peripheral parts of the nest in the early spring period.

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