Physiological Effects of Hypothermia on Queen Honeybees

Apis mellifera

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Received February 1, 2003

Abstract—Tolerance to and biological consequences of hypothermia of queen honeybees are studied. Sequence of their responses to hypothermia is traced and resistance to freezing is determined. There is revealed an effect upon reproductive function of cooling to the state close to freezing.

INTRODUCTION

Insects use diverse physiological ways of protection from cooling. In many species of solitary living insects, tolerance to hypothermia is determined mainly by the temperature of maximal supercooling, i.e., by its value, at which freezing of body liquid fractions begins [1–7]. For protection from freezing, the honeybee, like solitary living insect species, also uses biochemical means providing a decrease of temperature of the maximal supercooling [8, 9]. However, the dominating role in providing the cooling protection in the honeybee is played by etological mechanisms of regulation of intranest temperature [10]. Acquisition by honeybees of a complex instinct of the intranest temperature regulation has provided their families with high resistance to cold. During overwintering, they endure a decrease of external temperature to −50°C. However, the worker honeybees, when they are outside their nest, die at a temperature from −2°C to −16°C [8, 9]. As to sexually mature females (queens), they differ essentially by many physiological characteristics. The queens have a hypertrophied system of oogenesis, but there are absent many structures well-developed in worker honeybees, such as specialized wax glands and tools for collection of the blossom pollen. The queens exceed significantly the worker honeybees by their body mass, life span, and production of pheromones [10]. All this allows suggesting that hypothermia in queens can produce specific changes of the physiological state. The goal of the present study is to solve this task that is actual for understanding evolution of the social structure in the Apidae superfamily.

MATERIALS AND METHODS

The studies were carried out on inseminated and non-inseminated ovulating queens of the honeybee Apis mellifera. During hypothermia the age of the former was in the range of 1–15 days, of the latter, from 1.5 months to 2 years. The honeybee queens exposed to hypothermia were observed from several months to 3 years. In various variants of the experiments, 47 non-inseminated and 19 inseminated queens were used.

The experimental queens were cooled in a cold chamber till the state of cold torpidity or temperature of freezing. The temperature was controlled with an electrothermometer from the beginning of crystallization of liquid fractions in the cephalic, thoracic, and abdominal parts of the body. A microtether of the microthermometer was inserted into these parts cooled at a temperature maintained at the level of −18 ± 1°C [8].
In intact honeybee queens there was determined duration of the cooling, during which their cold torpidity occurred at a given temperature. In the torpid queens, at 24 ± 3°C, the beginning of activation was fixed. Using a stopwatch, there was determined duration of the periods of cooling, during which torpidity occurred, and that of warming—until the beginning of activation.

The non-inseminated queens exposed to hypothermia until the state of cold torpidity were allowed to mate. For this, they were placed to the queen-free honeybee families. After mating, their reproductive function was estimated by the number of laid eggs. The inseminated queens exposed to cooling were returned to their families. First the attitude of honeybees to them was observed, then their fertility was evaluated, and the state of their progeny was estimated by morphometrical parameters. For this, in each variant of the experiments performed in triples, in 30 born honeybees and in the same number of drones, wings, proboscis, and the 4th tergites were amputated. They were measured using an eyepiece micrometer of an MBS-10 stereoscopic microscope.

RESULTS AND DISCUSSION

The honeybee queens, like worker honeybees and drones, first responded to cooling by activation of locomotions, which is biologically appropriate under natural conditions when it is possible to escape from the zone of unfavorable temperature. Meanwhile, under conditions of isolation (the queens were in the cell, 22 cm³ in volume) this only increased duration of the period of cooling until the state of cold torpidity.

At 4 ± 1°C the high motor activity of the queens was observed for 7 ± 0.8 min. Then they stopped, but continued for 2–3 min to slowly raise and descend feet and to move with wings in the lateral plane. Only in 9.4 ± 1.1 min after the beginning of cooling the complete torpidity occurred. A decrease of the cooling temperature of the queens shortened duration of their activity preceding onset of the cold torpidity. Under effect of a decrease of temperature from 4 to 0°C, the period of their active state decreased 1.2 times \( P \) not lower than 0.99. The repeated coolings of the completely activated queens stimulated their similar reactions.

The torpid queens were activated under effect of warming. The activation duration depended directly on the duration of torpidity. Of essential significance was the temperature, at which the torpid queens became torpid and stayed in this state. If they were in torpidity at 2.5 ± 0.5°C for 15 min, they were activated at 26 ± 1°C for 6 ± 0.9 min. If the duration of the torpidity period increased to 30 min, the activation phase rose 1.5 times \( P \) not lower than 0.99. The queens in the state of torpidity at −2.5 ± 1°C for 15 min were activated for 9 ± 1.3 min. It took 13 ± 1.4 min for activation after the 30-min-long torpidity under the above conditions.

The honeybee queens died, if the cooling temperature reached the value, from which crystallization of body liquid fractions started. This temperature differed in the content of the cephalic, thoracic, and abdominal parts and differed with aging of the queens. In young queens aged 1–3 days, the content of the head froze at −5.1 ± 0.81°C, of the thorax, at −4.4 ± 0.72°C, and of the abdomen, at −4.1 ± 0.69°C, whereas in the 1.5 ± 0.5-day old queens, this temperature was lower 1.8, 1.6, and 1.4 times, respectively \( P \) higher than 0.99.

Since the young non-inseminated queens and the ovulated queens of different ages differ by their resistance to freezing, to evaluate delayed consequences of hypothermia, they were maintained in the torpidity state at different temperatures. In the former, its minimal values were higher by 5.5°C than in the latter. The exposition duration in the cold torpidity state was 1 h.

The young honeybee queens exposed to hypothermia at −0.5 ± 0.5°C, after several days of stay in the queen-free honeybee families, mated and began to lay the fertilized eggs, from which worker bees developed; these did not differ from the mean norm by their morphometrical parameters. The cooled queens did not differ statistically significantly by their fertility from the queens that were not exposed to cooling. Either queens, in 2–3 weeks after insemination, laid daily 720–790 eggs (determined by the number of queen cells of the closed brood). Cooling of queens somewhat affected their preservation at the 5–8-day long period of their flights to mate. The losses among the cooled queens were higher by 14% than among the non-cooled ones \( P \) lower than 0.9.

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