DOI: 10.2478/v10289-012-0012-z
Vol. 56 No. 1 2012 Journal of Apicultural Science VERSITA

SUPERCOOLING POINTS OF *APIS MELLIFERA LIGUSTICA* WHEN PERFORMING DIFFERENT AGE-RELATED TASKS

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Received 04 January 2012; accepted 05 March 2012

S u m m a r y

In order to study the cold resistance of honey bees, the authors systematically investigated the supercooling points (SCPs) of *Apis mellifera ligustica* worker bees performing different age-related tasks. There were statistically significant differences in SCPs between worker bees performing different activities ($P<0.05$). The nectar-water collector had the highest SCP temperature (-3.16°C), highest water content (74.85%) and lowest crude fat content (6.13%). The soldier had the lowest SCP temperature (-6.40°C), relatively lower water content (70.33%) and higher crude fat content (7.28%). No significant difference was found in the SCPs of workers of different ages. Winter bees did not differ from summer bees in their SCPs. The relatively higher SCPs from different kinds of individual bees suggest that honey bees do not mainly rely on their low SCPs for their cold resistance.

Keywords: honey bee, *Apis mellifera ligustica*, workers, supercooling point, water content, crude fat content.

INTRODUCTION

Insects that are cold-hardy but sensitive to freezing, survive at low temperatures due to their supercooling capacity. In some species, supercooling points (SCPs) are below -25°C (Zachariassen and Husby, 1982). In freeze-avoiding insects, all potent ice-nucleating agents are removed or inactivated, leading to a depression of a SCP to below -20°C. The regulatory mechanisms of supercooling are influenced by environmental factors, like photoperiod and temperature (Zachariassen, 1985). Nevertheless, it is widely accepted that SCP alone can not be considered as a single, sufficient descriptor of insect cold tolerance. Honey bee (*Apis mellifera*) colonies maintain stable brood nest temperatures of 33-36°C (Seeley, 1985). When gathered in large clusters, bees maintain a central core temperature of 34°C even during exposure to extreme cold air temperatures down to -80°C (Southwick, 1987). At low ambient temperatures, the bees crowd in the brood area (Kronenberg and Heller, 1982; Harrison, 1987) and produce heat by vibrating their thoracic muscles without moving their wings (Esch et al., 1991; Heinrich, 1993; Heinrich and Esch, 1994). High ambient temperatures are counteracted by wing fanning and water intake for evaporative cooling (Hazelhoff, 1954). Concerning honey bee overwintering, it is interesting to know whether or not they show acclimatization to subzero temperatures via a change of their supercooling temperature. For worker bee cold-hardiness, both physiological and ethological explanations are important. For example, workers form a tight cluster to resist the cold in winter, foragers sometimes have to face changeable weather when they go out foraging, guards patrol at a temperature fluctuating entrance (Fahrenholz et al., 1989), and in a cold weather soldiers fly out from the "warm" hive for defending purposes etc. In this paper we investigated the SCPs of adult *A. m. ligustica* workers with respect to age, task performance, season and with respect to the water and lipid content of their bodies. This investigation will help in the understanding of whether or not the SCPs of different bees are related to their cold resistance, and to show the relationship between SCPs, water and lipid content. Besides, It will show if there is any difference in the SCPs of worker bees of *A. m. ligustica* compared to *A. cerana cerana* and *A. m. carnica*.

MATERIALS AND METHODS

Study site and bee colonies

The experiments were done from June of 2007 to June of 2011 at the China Agricultural University, Beijing (39°92'N,116°46'E), using the honey bee *Apis mellifera ligustica*. The colonies were maintained according to routine techniques. Each colony was composed of 15-20 combs of bees.

Instruments and testing methods for the testing of SCPs

The supercooling testing method was that used by Qin and Yang (2000). A thermistor-avometer (electric multimeter) assembly was employed to test the SCPs of the adult honey bees (Fig. 1). The thermistor (TH-C35) and avometer (M-3800) were made in China (Shen Zhen Hua Yi Experimental and Test Meter Co., Ltd.). The relationship between the resistance (Ω) and the temperature (T) (°C) was described by the following equation:

$$T = 10^{2.8158 - 0.5438941g\Omega} - \Omega / 40 - 29$$

The bees were not chilled before testing. One bee at a time was put into the testing tube (made of a 1 mL pipet tip). A ball of cotton was put into the tube after the bee had been put in to make sure the bee had a tight contact with the thermistor. The resistance value (kΩ) increased with the decreasing body temperature of the tested bee. When the body of the bee began to freeze, a sudden decrease of the resistance (i.e. an increase of temperature) was noticed, due to the release of crystallization heat. The highest resistance value before the “sudden decrease of the resistance” was the SCP.

Labour division of adult workers

From the middle of July to the middle of August 2007, the following categories of adult worker bees were collected: nurse bees, guards, soldiers, nectar-water collectors and pollen collectors. All the bees were sampled from three colonies between 9:00 a.m. and 11:00 a.m. Ten bees from each category were collected from each colony and their SCPs were tested immediately. Each category of workers was defined as follows (Yang et al., 2007): Nurse - a bee with its head and part of its thorax inside a brood cell, Guard - a patrolling bee at the hive entrance, Soldier - a bee flying out and attacking a provoking experimenter, Nectar-water forager - a forager carrying nectar or water in its crop alighting at the hive entrance, Pollen forager - a forager returning to the hive with pollen loads in its corbiculae.

Age of adult workers

From the middle of July to the middle of August 2007, three combs each from one hive were collected which had pupae with black pigment in their eyes. The combs were put into a dark incubator for the pupae to emerge at conditions of 35°C and 60-70% relative humidity (RH). Forty-eight hours later, when the emerged workers were two days old, 10 bees were collected, and their SCPs were tested immediately. Further samples made up of 10 bees, were tested when the bees reared in the incubator were at age 4, 6, 8, 10, 12, 14, 16, 18 and 20 days. Each bee was tested only once at one age.

Seasonal Changes in adult workers

From July of 2007 to May of 2008, 10 nurse bees (Jul, Aug, Sept, Mar, Apr, May) or 10 in-hive worker bees (Oct, Nov, Dec, Jan) were collected on the twentieth day of each month and their SCPs were tested. The bees from three colonies were tested from July to December in 2007. Unfortunately, two colonies collapsed in January of the next year, so data from only one colony were used.

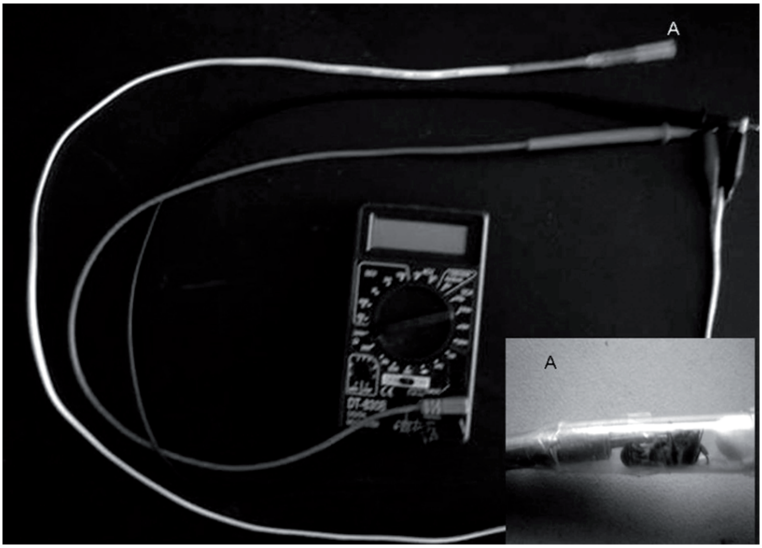

Fig.1. Thermistor - avometer assembly, and an adult worker bee *Apis mellifera ligustica* connected to the thermistor (see A).

Testing of water content

A test was carried out in April 2011 for understanding the correlation between water content and SCPs. Five kinds of workers, the same as above, were sampled from three colonies, 5 workers / each kind / colony, repeated 5 times. The workers were weighed, then dried at 60°C (48 h) to a constant mass (to unload the pollen loads before drying for the pollen foragers) and weighed again. The last thing done was to calculate the water content (% fresh mass) of each sample.

Testing of Crude Fat Content

In the middle of October in 2011, five categories of adult workers, the same as above, were collected. All the bees were sampled from three colonies between 9:00 a.m. and 11:00 a.m. Five kinds of workers, were sampled from three colonies, 25 workers / each kind / colony, repeated 3 times. The bee samples were dried to a constant weight in 70°C and then ground into powder (to unload the pollen loads before drying for the pollen foragers). Each time, about 0.5 gram of the powder (200 mesh) was extracted in a Soxhlet extractor (100ml) with petroleum ether (b.p. 30-60°C) by a thermostat water bath at 55°C for 11h. The residue was discarded. The solution was concentrated to about 15mL and dried in an oven at 105°C until it was at a constant weight to obtain crude fat extract. The extraction was repeated three times.

$$\textit{Crude Fat}\ (\%) = \frac{\textit{Dried Crude Fat Extract}\ (\mathrm{g})}{\textit{Sample Powder Weight}\ (\mathrm{g})} \times 100\%$$

Statistical analyses

ANOVA was employed to determine the statistical differences in the SCPs or water contents between different treatments. Tests used for normality and homogeneity of variance were done before ANOVA. Tukey's HSD post hoc multiple comparisons was performed after ANOVA to reveal significant differences between samples.

RESULTS

SCPs of workers performing different age-related tasks

There were statistically significant differences in SCPs between the three colonies ($F=3.78_{2,135}$, P=0.025). Further statistical analysis showed that the differences came from the data about the nectar or water collectors ($F=11.47_{2,27}$, P<0.01). The average SCPs of nectar-water collectors from three colonies were -2.81°C, -2.92°C and -3.77°C, respectively. There were statistically significant differences in SCPs between bees performing different activities ($F=141.16_{4,135}$, P<0.01; Fig. 2). Among the five groups, soldiers had the lowest SCP (-6.40°C) and nectar-water collectors had the highest (-3.16°C). There was no significant difference between nurse and pollen foragers and also no significant difference between guard and pollen foragers. The SCP ranking was: soldier<nurse<pollen collector<guard<nectar-water collector.

SCPs and age

There were no statistically significant differences in the SCPs of different ages (1 to 20 days) ($F=1.01_{9,90}$, P>0.05). The lowest SCP was in 8d workers (-5.3°C) and the highest in 4d workers (-4.25°C).

SCPs and season

There were no statistically significant differences in the SCPs of adult workers collected monthly from July 2007 to May 2008 ($F=0.89_{9,90}$, P>0.05). The lowest SCP was seen in December (-5.2°C) and the highest in March (-4.3°C). The mean SCP of overwintering workers from Oct, Nov, Dec, Jan was -4.84 °C (-4.32°C - 5.20°C).

Water content of different workers

There were no statistically significant differences in water content between the three colonies ($F=0.38_{2,26}$, P=0.69), so the data of the three colonies were combined. The water contents differed significantly between the group of nectar-water collector and the other 4 kinds of workers ($F=51.03_{4,60}$, P<0.01). The water content ranking was: nectar-water collector>pollen collector>soldier>guard>nurse. There were no significant differences between the pollen collector, soldier, guard or nurse categories (Tab. 1).

Crude fat content of different workers

There were statistically significant differences in crude fat contents between the three colonies ($F=34.45_{2,30}$, P<0.01). The ANOVA procedure was done separately on the colonies, and the results showed a same tendency of the crude fat

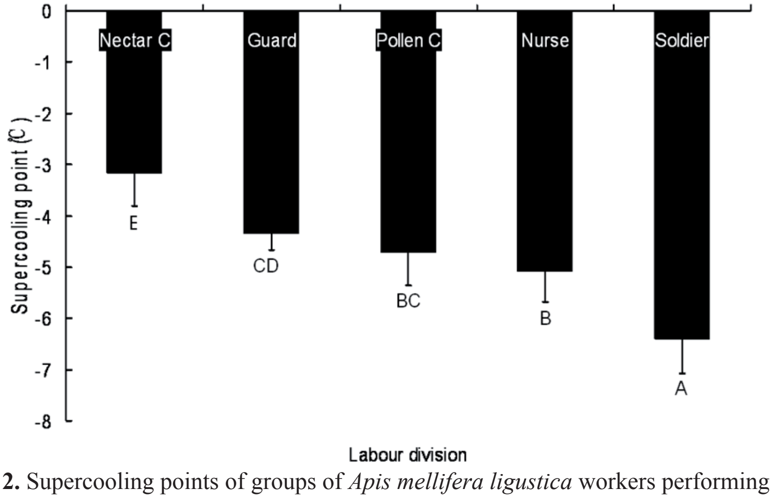

Fig. 2. Supercooling points of groups of *Apis mellifera ligustica* workers performing different tasks. For labor divisions see text (Nectar C = Nectar-water forager; Pollen C = Pollen forager). The bars represent mean ± SD. Statistical differences (P<0.01) are indicated by different letters.

content: nurse>guard>soldier>pollen collector>nectar-water collector. The combined ANOVA from three colonies showed that the crude fat contents differed significantly between the nurse and the other 4 kinds of workers ($F=116.68_{4,30}$, $P<0.01$). There were also significant differences in crude fat contents between the nectar-water collectors and pollen collectors, soldiers and guards, however there were no significant differences between pollen collectors, soldiers or guards (Tab. 1).

Table 1.

Water and crude fat content of different groups of workers performing different tasks

	Nurses	Guards	Soldiers	N.-W. Collectors	P. Collectors
W. Content (%)	68.61±1.87B	69.90±0.78B	70.33±1.68B	74.85±2.57A	70.45±1.20B
Crude fat (%)	8.99±0.48A	7.62±1.06B	7.28±0.60B	6.13±0.65C	6.74±0.79BC

Note: P. Collectors ~ pollen collectors; N.-W. Collectors ~ nectar-water collectors; W. Content ~ water content. The Data in the table are mean ± SD. The data followed by different capital letters in the same line are significantly different at the 0.01 level.

Comparatively, the nectar-water collectors had both the highest SCP temperature and water content, but the lowest crude fat content. The soldiers had the lowest SCP temperature. Both the water content and crude fat content of the soldiers were not significantly different when compared to the pollen collectors and guards. The nurses had the highest crude fat content, and a relatively lower SCP and water content.

DISCUSSION

Danks (1996) reported that water content and bound water influence the SCPs of insects. The abdomen of nectar-water foragers which are filled up with nectar or water might have reduced their cold resistance compared to the other workers. Our result showed water content could influence SCPs of the nectar-water collectors, the higher the water content - the higher the SCP. But the results did not show any regularity between the SCPs of nectar-water collectors and the water contents for the other kinds of workers. We pooled both water and nectar foragers into one group because it was difficult to distinguish them in the short time they were at the hive entrance. Lipid content has a close relationship with the cold resistance of insects (Zhao et al., 2010); the more crude fat content of *A. m. ligustica,* the higher the cold hardiness (Chang et al., 2007). Keller et al.(2005) showed that fat body of nest bees was more developed compared to forager bees. Their results were in agreement with our results: the nurses had a relatively higher crude fat content, and nectar-water collectors had a lower level. Specific hormones control various processes, including elements of cold hardiness, such as juvenile hormones which help control "cryoprotectant" production, and also nucleators (Danks, 1996). Honey bees, though, are not known to produce cryoprotectants. A considerable part of the task allocation of bees is related to age. Yet in our results, there were no significant differences in the SCPs of different aged adult worker bees. This may be caused by what is called the "keeping condition". This is the condition in which different aged bees caged in an incubator do not work and can not develop normal work divisions as normal bees do in the hive. The SCP of overwintering workers is -4.84 °C, which is higher than from *A. c. cerana* (-6.9°C) or *A. m. carnica* (-5.9°C) (Li et al., 2006). This shows that western honey bees have a relative lower cold-hardiness. Winter bees do not differ from summer bees in their SCPs. This is possible because cooperative metabolism of honey bees during overwintering is an alternative to the antifreeze and hibernation strategies of other insects (Southwick, 1987). Honey bees live in a big colony, at low ambient temperatures.

They are protected against freezing mainly by their group behavior, but not by individually changing their bio-chemistry. Chen et al. (2001) showed that there is a close relationship between bee activity and their environment. A relative study showed the average body temperature of honey bees (*A. m. carnica*) in spring was higher than in summer. The higher body temperatures in spring possibly protect the bees against cooling at the lower ambient temperature (Kovac and Schmaranzer, 1996). Although bees have a social mechanism of thermoregulation, the cold-hardiness is crucial for individual bees if they sit at the surface of a winter cluster. If they fall off the cluster (as bees sometimes do), they may be exposed to subzero temperatures. We briefly examined the SCPs of different worker bees, considered their cold-hardiness at two levels of their organization: the individual and the colony level. The latter is sometimes termed a 'superoganism' (Moritz and Southwick, 1992). To examine the individual bee from a winter cluster, can help us understand the character of cold resistance for the whole bee colony through one aspect, even though the bees 'as a whole' are able to generate substantial heat to maintain the temperature of the over-wintering clusters. Watmough and Camazine (1995) pointed out that thermoregulation is a result of the collective actions of the individual bees. The SCPs found in the present investigation demonstrate that social thermoregulation keeps honeybees at a sufficient thermal distance from freezing hazards throughout the winter. The absence of antifreeze measures underscores the widely accepted opinion that *A. mellifera* is primordial, a tropical insect which was able to conquer temperate and cold regions mainly because of social behavior. Nevertheless, individual worker bees have some need for cold-hardiness during the breeding season. For example, the foragers go out foraging in the changeable weather of early spring or late autumn, in early morning or late evening etc. Guards work in an unstable ecological environment; the temperatures at the hive entrance may fluctuate widely (Fahrenholz et al., 1989). So, these facts are conducive for testing the cold-hardiness of foragers and guards, although guarding and foraging activities are not performed during the cold winter. Other social insects like wasps (*Vespula*, *Vespa*, …) develop a different strategy to survive the cold: social thermoregulation in summer and physiological adaptations to achieve freeze tolerance in winter. Many overwintering insects become cold hardy due to the photoperiodic induction of diapause, which is a prerequisite for the later effect of low temperature (Hodková and Hodek, 2004). These insects can be protected individually against freezing mainly by bio-chemistry (Zachariassen and Husby, 1982; Zachariassen, 1985; Shimada, 1989).

CONCLUSIONS

Our results show a statistically significant difference in SCPs between worker bees performing different activities. The nectar-water collectors had the highest SCP temperature, the highest water content and lowest crude fat content. The soldiers had the lowest SCP temperature, a relatively lower water content and a higher crude fat content. No significant differences were found in the SCPs of workers of different ages. Winter bees did not differ from summer bees in their SCPs.

ACKNOWLEGEMENTS

We sincerely thank Di Wu, Yijiao Wang, Chunyan Liu, Qian Zhao and Yuhang Wu from the Department of Entomology, China Agricultural University for the water test of honey bee workers.

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Journal of Apicultural Science

PUNKTY PRZECHŁODZENIA PSZCZÓŁ *APIS MELLIFERA LIGUSTICA* WYKONUJĄCYCH RÓŻNE PRACE ZWIĄZANE Z WIEKIEM

Li Y., Wang S., Qin Y.

Streszczenie

W celu zbadania wytrzymałości pszczół na niską temperaturę, autorzy pracy badali punkty przechłodzenia (SCPs) pszczół robotnic *Apis mellifera ligustica* wykonujących różne prace związane z wiekiem. Stwierdzono statystycznie istotne różnice w SCP pomiędzy robotnicami wykonującymi różne prace. Zbieraczki nektaru i wody miały najwyższą temperaturę SCP (-3,16°C), największą zawartość wody (74,85%) i najniższą zawartość tłuszczu w ciele tłuszczowym (6,13%). Strażniczki miały najniższą temperaturę SCP (-6,40°C), niższą niż zbieraczki zawartość wody (70,33%) i wyższą zawartość tłuszczu w ciele tłuszczowym (7,28%). Nie stwierdzono istotnej różnicy w SCP wśród pszczół robotnic różnego wieku. SCP pszczół zimowych nie różnił się od SCP pszczół letnich. Relatywnie wyższe SPC u pojedynczych pszczół wskazuje, że odporność pszczół na zimno nie zależy głównie od ich SCP.

Słowa kluczowe: pszczoła miodna, *Apis mellifera ligustica*, robotnice, punkt przechłodzenia, zawartość wody, zawartość tłuszczu.