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Cold Resistance of the Small Carpenter Bee *Ceratina flavipes* Restudied[1]

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Abstract The overwintering adults of the small carpenter bee *Ceratina flavipes* is freezing susceptible as reported previously. Its supercooling points fluctuate from −16°C to −28°C. From this range and distribution of the species in S. E. Hokkaido, being present in Tokachi Province but so far unrecorded from lowlands of Kushiro Province, the latter fact is interpreted as caused by climatic conditions in the active season rather than the winter severity. A high monosaccharide level in the overwintering adults reported previously depends on the crop contents (fructose 9.8%, glucose 12.2% of crop weight), not on sugar contents in other body parts. On the other hand, the trehalose level in the latter is fairly high, occupying 3.4% of body weight. It is probable that both these sugar levels enhance the supercooling ability of the overwintering adults.

Introduction

In a previous paper, one of us reported high monosaccharide contents (mainly glucose and fructose) in total homogenate of two small carpenter bees, *Ceratina* (*Ceratinidia*) *flavipes* Smith and *C.* (*C.*) *japonica* Cockerell, and assumed their significance in enhancing the supercooling ability (10). However, the adults emerged in the autumn of these bees visit flowers and ingest nectar before entering the hibernacula (7). In the halictine bees, which possess the same habit, high sugar levels in their crops are recognized (8). It is likely that higher sugar levels found in *Ceratina* also depend in part on their crop contents, not on sugars contained in haemolymph. It is generally accepted that the cuticle-lined foregut of insects is not or little permeable to most substances, including water and sugars (15, 16). Then the relation between sugar levels and cold resistance might vary according to whether the concentrated sugars are deposited in the crop or haemolymph. In the present study the sugar contents were determined for crop and other body parts separately. Further, in the previous study (10) the cold resistance was expressed by survivals at four successive subzero temperatures. Direct measurements of the supercooling points (SCP) were undertaken in this study.

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Materials and Methods

Materials: The overwintering adults were collected on January 3, 1979, at the suburb of Sapporo from dead but erected stalks of *Artemisia* used by bees as hibernacula. At collecting, the bees were found at the level about 30 cm above the ground, where the stalks were exposed above the snow surface. The bees were preserved in a freezer kept at −5°C until studied in mid February, 1980.

Measurements of SCP: Each bee was coated with cotton wool and covered in triple with a gelatin capsule (6 $\phi \times 17$ mm), a glass vial (3 $\phi \times 8$ cm) and a plastic vial (6 $\phi \times 10$ cm). The tip of a thermocouple was inserted into a space between bee and cotton wool. The thermocouple was connected with a recorder (Rikadenki KB681H) and the whole set was cooled in a freezer with the cooling rate of about 0.5°C/min. SCP were determined from the cooling curves recorded.

Chemical analysis: The bees with which SCP were determined were also used for chemical analysis. After measuring the body weight, some bees were soon fixed with 80% ethanol. In other bees, the body was separated at the junction between propodeum and metasoma, which could be made with little loss of body fluid from the fore body thanks to the structural peculiarity of the higher Hymenoptera. The fore body was weighed and fixed with ethanol. The metasoma was dissected in water, the crop was removed, weighed and fixed. The materials were kept at −25°C till analysed as follows.

Each sample was homogenized with 0.1 ml internal standard, erythritol solution (10 mg/ml), in 2 ml of cooled (3°C) 80% ethanol, and centrifuged at 1,500 *g* for 10 min. After removing precipitation, the supernatant was evaporated at 50°C under a reduced pressure 10～20 min. The residue was dried under a reduced pressure over P_2O_5 overnight. To the dried sample 0.2 ml TMSI-C (Gasukuro-Kogyo, Tokyo) was added and heated at 65°C for 1～4 hrs to prepare the trimethylsilated derivatives. The obtained derivatives were applied to gas-liquid chromatography (Shimazu gas chromatograph, GC-4CM) using a glass column, 3 m × 3 mm packed with 1.5% OV-1 on chromosorb W. The chromatograph was run by the program of ascending temperature from 130°C to 270°C. The temperature was raised at the rate of 5°C/min and held at 270°C for 10 min.

Results

Supercooling points

The measured volumes of SCP varied considerably, showing a somewhat bimodal distribution (Tables 1 and 2). Such variability in the cold resistance is also suggested in the previous study (10) in which two species were indiscriminably tested. The present result suggests that fluctuation in the previous

Table 1. Fluctuation of supercooling points in the overwintering adults of *Ceratina flavipes*

SCP (−°C)	≥16	≥18	≥20	≥22	≥24	≥26	≥28
♂		4	1	1			5
♀	1			2		2	5
♂+♀	1	5	1	3		3	8

Table 2. Mean supercooling points (−°C) in the overwintering adults of *Ceratina flavipes*

Sex	Range	$\bar{x}$	SD	n
♂	16–22	18.1	1.49	6
	26–	26.9	0.54	5
	Whole range	22.1	4.55	11
♀	16–22	18.9	2.53	3
	26–	26.5	0.84	7
	Whole range	24.2	3.78	10
♂+♀	16–22	18.4	1.94	9
	26–	26.6	0.76	12
	Whole range	23.1	4.33	21

result did not depend on the specific difference. No significant difference was found between two sexes. In this species, some overwintering females, e. g. about 13% in Morioka, are those having entered in their second hibernation (7). The female who showed the highest SCP (−15.4°C) was such a two years old female once had participated in brood rearing activity. Whether the cold resistance differs between young and two years old females or not should be clarified in future.

As in the previous study (10) no frozen adults could survive after thawing. They exhibited a characteristic posture, extending the antennae. In five females the sting was protruded. Seventeen out of 21 tested adults tightly bit cotton wool fibers with mandibles. One male and one female regurgitated a yellowish brown liquid on fibers, probably secreted from the mandibular glands. As SCP of these individvals were fairly low (−26.3°C, −27.2°C), the ice inoculation through this liquid is unlikely. SCP values are comparable to the higher levels exhibited by other insects overwintering in natural stalk stub (12).

In some adults both body and crop weights were measured (Table 3). Mean body weight is distinctly larger in females but percentage ratio of crop/body weight does not significantly differ between the two sexes, ranging 3.3～11.7% in females and 4.3～15.4% in males. An appreciable amount

Table 3. Body and crop weights in the overwintering adults of *Ceratina flavipes*, measured in the same individuals (in mg, $\bar{x}$+SD)

Sex	Body weight	Crop weight	% Crop/Body	*n*
♀	15.64±2.08	1.30±0.48	4.7	6
♂	11.26±1.84	1.08±0.46	5.3	6

Table 4. Sugar contents in the overwintering adults of *Ceratina flavipes* (% weight ±SD) Contents in fore body and crop were measured in the same individuals. (—=trace)

Sugar	Fructose	α Glucose	Trehalose	*n*
Whole body	2.2±0.8	4.2±1.5	4.5±0.3	4
Fore body	—	—	3.4±0.8	16
Crop	9.8±4.7	12.2±5.0	—	11

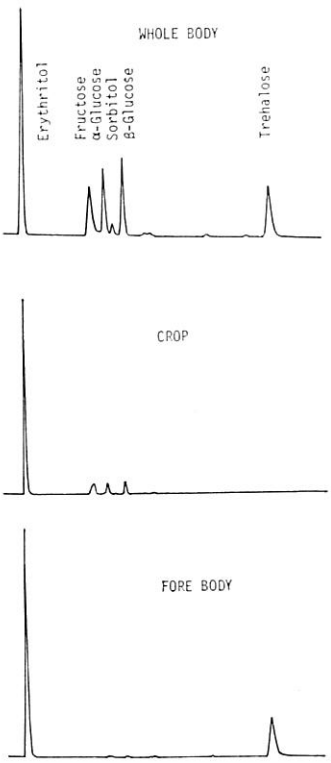

Fig. 1. Gas-liquid chromatograms of sugar contents in the overwintering adults of *Ceratina flavipes*. Crop and fore body from the same bee

of solid food residue was detected in the hind gut of some adults but not in the crop and mid gut. All females possessed thread like ovaries. Their fat bodies had more developed than in some halictine bees overwintering in soil (6).

Sugar contents

Comparison of the three kinds of samples, whole body, fore body and crop (Table 4 and Fig. 1), clearly demonstrates that glucoses and fructose were detected nearly exclusively in the crop, not in the fore body. Apparently these sugars were inverted by the adult bees from sucrose, the main component of sugars found in flower nectar. Trehalose was virtually absent in the crop but showed a fairly high level in the fore body, probably mostly in haemolymph, being comparable to the overwintering stage of some insects, e. g. the poplar sawfly *Trichiocampus populi* (11) and the saturniid moth *Philosamia cynthia* (2). A trace of sorbitol was detected in two adults (Fig. 1, whole body), but glycerol was found in no samples.

Discussion

The genus *Ceratina* is principally a warm climate group and the subgenus *Ceratinidia* is typically Indomalayan (3). But two northernmost representa-

tives of this subgenus *C. flavipes* and *C. japonica* are fairly common in Hokkaido, not only in the western part but also in Tokachi Province. In a periodical sampling of wild bees made in Obihiro, Tokachi, *C. flavipes* occupied the top rank in relative abundance (14). Judging from the range of SCP reported above, the winter survival may be seldom threatened by low temperature in W. Hokkaido, especially considering the thick snow cover developed there. However, the influence of thermal factor upon the winter survival is not precluded in Tokachi Province, where the minimum air temperature not rarely attains $-20°C$ or less and the snow cover is often relatively thin till the mid winter, making protection of *Ceratina* hibernacula incomplete. Although further studies are required, escape into particular microhabitats by adult bees before overwintering is possible.

Up to the present, both species are unknown from the lowlands of Kushiro and Nemuro Provinces, the easternmost Hokkaido (13), nevertheless the winter thermal severity is not much high there than in Tokachi : mean annual minimum air temperature in Obihiro and Kushiro is $-16.2°C$ and $-13.0°C$ and the sum of mean monthly subzero temperatures $-24.4°C$ and $-23.6°C$ respectively. Possibly the absence of the two species in the lowlands of Kushiro and Nemuro is realized by adverse thermal conditions there in active seasons (warmth index 46.9°C in Kushiro against 59.0°C in Obihiro). Moreover, the scarcity of fine days in Kushiro (number of foggy days during May to September 80.8 against 41.7 in Obihiro) should affect the foraging activity of the two species, which are extremely heliophilous, leaving nests only on fine days.

The present study proved that high levels of glucose and fructose reported previously depend mostly on the crop contents. The same could be expected for the bee groups which overwinter in adult stage after autumnal feeding i. e. the bumblebees (queens alone) and halictine bees (in most species females alone, cf. 5). Certainly these sugars are consumed mainly in the late autumn and early spring within hibernacula, as the winter metabolism should be quite low.

In the carpenter ant *Camponotus obscuripes*, two SCP are detected in each supercooling curve, the first SCP at $-8.5\pm0.3°C$ with less heat release and the second one ranging from $-10.3\sim25.6°C$ with higher heat release (4). From the recovery of ants rewarmed before attaining the second SCP and the results of differential thermal analysis and freeze-sectioning, as well as from the so far accepted impermeability of cuticle-lined insect fore gut, it was assumed that the first SCP corresponded to freezing in the fore gut and the second SCP to that of the entire body (4). Despite the morphological similarity of its fore gut to that of ants, such first SCP with small heat release was not observed in *C. flavipes*. The tested adults were cooled down to $-30°C$, but all freezing curves showed a single heat release. This suggests that freezing spread continuously, irrespective of where it had started, either in the fore gut, mid- or hind gut or in other body tissues.

In the worker honeybee the proventricular valves possess an efficient ability in separating solid particles in the fore gut from the medium and sending them into the mid gut. The particles of 3 μ or less (*Nocema* spores) to 16～30 μ (pollen) are packed and passed into the mid gut within 20～25 min (1). The same function is also expected in *Ceratina*. But this ability may be inefficient in separating smaller natural motes with sizes of mμ or less, which are taken through the mouth and serve as ice nucleators. Thus, the fore gut is considered a site initiating freezing more easily than other body tissues. Further, the proventricular valves may be able to separate the contents of fore and mid gut but not so efficiently to prevent the spread of freezing from the former to the latter. However, this higher chance of freezing is probably counteracted by the high monosaccharide level found there. On the other hand, the trehalose level in other body parts, probably in haemolymph, is fairly high in *Ceratina* though obviously less than the monosaccharide level in the fore gut. It is probable that this enhances the supercooling ability to some degree. Thus, the two body sections, the fore gut and other parts may behave differently confronting freezing, the former with a higher chance of freezing but with a higher sugar level and the latter *vice versa*. From the continuous proceeding of freezing in *Ceratina*, it is unknown where freezing is initiated. But irrespective of the site of initiation, the higher suger levels in the two body sections may contribute in lowering SCP of the entire body.

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