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SEXUAL DIFFERENCE OF COLD-HARDINESS IN OVERWINTERING LARVAE OF GIANT BAGWORM MOTH

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Introduction

Ice formation within the bodies of freezing-susceptible insects is fatal. However, since in most of these supercooling is not necessarily dangerous, the supercooling point represents the lethal limit of low temperature (Salt 1961). From an ecological point of view, the ability of freezing susceptible insects to supercool is of great importance to the geographic distribution of the species.

There are many factors influencing supercooling, nucleation and ice growth. Salt (1966) has presented a discussion of factors influencing nucleation in supercooled insects. So far, there has been little work done on sexual differences in supercooling ability or on the effect of insect size on supercooling, because there are few appropriate materials. In this respect, overwintering larvae of the giant bagworm moth are suitable material as the sexes differ in size, color, behavior and so forth.

In the present paper, the supercooling points and percent body moisture of larvae of both sexes were determined from fall to spring and their interrelations were investigated. The abilities of survival and supercooling at -13.5C, and the sexual difference in water loss and survival at a constant temperature of 2 C, as well as outdoors during winter were also investigated.

Materials and Methods

Mature larvae of the giant bagworm moth, *Clania variegata*, overwintering on the twigs of acacia (*Robinia pseudo-Acacia*) in Fukuoka were used as material. From the fall of 1964 to the spring of 1965 the changes in supercooling points and percent moisture of larvae were measured in both sexes. Supercooling points of intact larvae were measured by a copper-constantan thermocouple (0.25 mm in diameter) connected to a galvanometer (TOA Electronics AD-7) and a polyrecorder (TOA Electronics

EPR-2T). The tip of the thermocouple was kept in contact with the surface of the abdomen. To slow down the rate of cooling the assembly was enclosed within two concentric glass vials before being placed in a freezer. The cooling rate was about 1.5 to 2.5 per minutes at 0 C, decreasing from this value as the temperature fell. Percent body moisture of larvae was determined by drying the larvae at 90 C for 24 hours and expressed on a fresh weight basis. Determination of glycerol content was made chromatographically with the method described by Somme (1964). The larvae used for glycerol analysis were collected in February of 1966 at Fukuoka, and analysis of glycerol was carried out at the Research Station, Canada Department of Agriculture, Lethbridge, Alberta during my tenure as Postdoctorate Fellow of the National Research Council of Canada, 1965-66.

To determine the ability of supercooling and survival at -13.5 C, 100 larvae of each sex were placed individually in small plastic vials and exposed to -13.5 ± 1.0 C. Ten larvae of each set were removed from the freezer at appropriate time intervals and the state of larvae whether frozen or not was examined. The survival of larvae was determined by response to gentle prodding after three days at room temperature.

Water loss (decrease of body weight) in winter was measured as follows: 160 larvae of each sex were individually weighed in November, 1967. When the larvae were replaced in their cocoons, they repaired them in a few days. Eighty cocoons of each sex were then placed outdoors and 80 were exposed to a constant cold temperature, about 2 C. The body weights of 20 larvae of each set were taken monthly and the ratio to the initial weight of each was determined. Survival of larvae was counted on the same materials.

Results and Discussion

Sexual differences in supercooling points and relation to body weight and water content

The sexual differences in body size and weight are a marked characteristic of this species. As shown in Fig. 1, the weights of male larvae ranged from about 250 to 500 mg and female larvae from 450 to 1800 mg. That is, females are about two or three times heavier than males. In November and January the supercooling points of male larvae were about -15 to -20.5 C, and of female larvae -11 to -17 C. Male larvae have larger supercooling ability by about 4 C than female ones (Fig. 1). Siegler (1946) found that the undercooling temperatures of the larvae of hibernating codling moth were practically unaffected by the sex or weight of larvae. However, the sexual differences of size or weight in the larvae of codling moth were very slight as compared with those of the giant bagworm moth.

The relation between water contents and supercooling points was also examined. As shown in Fig. 2, the total water content does not show

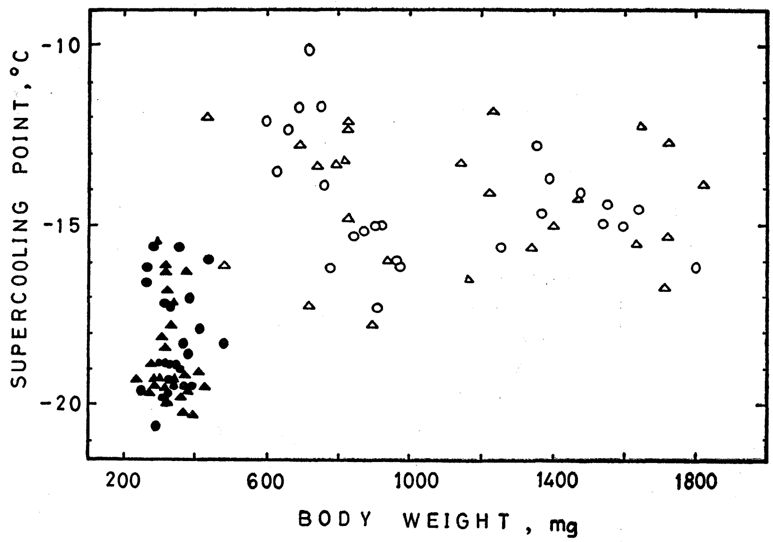

Fig. 1. Relation between supercooling points and body weights of larvae of both sexes. ● male, ○ female larvae in November, ▲ male, △ female larvae in January.

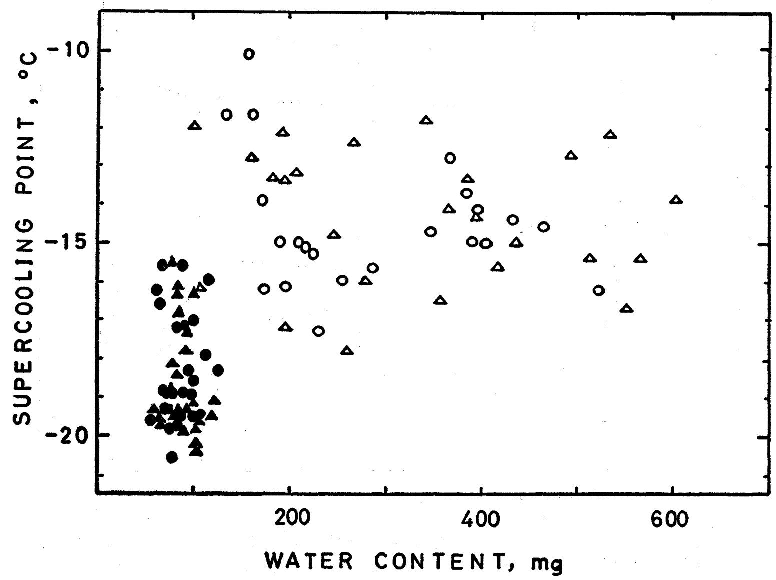

Fig. 2. Relation between supercooling points and total water contents of larvae of both sexes. Symbols as in Fig. 1.

any definite relation to supercooling ability. The inverse relation between volume and supercooling points of water or liquid is to be expected. Neverthless, there is no definite relation between body weight, water content and supercooling point *within the same sex.*

A volume effect, reflecting the probability that a larger volume of water contain more and better nucleators than does a small volume, may be applicable to the freezing of biological material. In this respect, the quantity of water experessed as to total water content is more important than the percent moisture. As seen in Fig. 2, however, it seems unlikely that the larger the total water content the higher the supercooling points. As a result, the volume dependence of supercooling points could not be

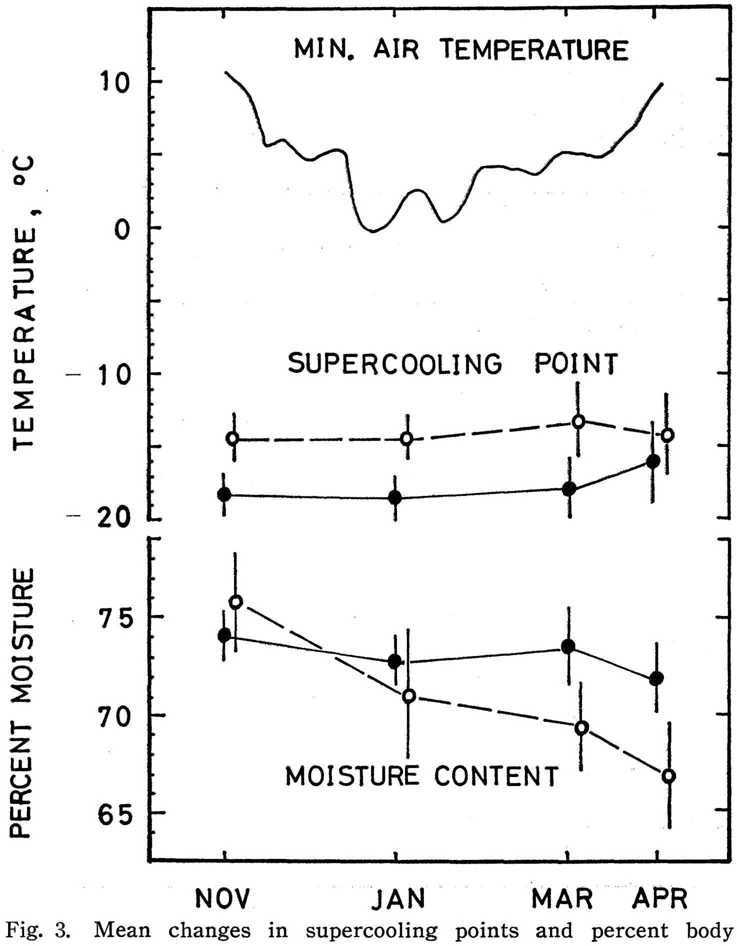

Fig. 3. Mean changes in supercooling points and percent body moisture of larvae of both sexes (● male, ○ female), and seasonal changes in minimum air temperatures during winter in 1964-65. Vertical bars represent standard deviations.

detected in larvae of the same sex. This is in marked contrast to recent work using plant materials; the supercooling points of conifer needles were an increasing function of each of their length, weight and water content (Kaku and Salt 1968). The haemolymph in the bulk space of the cavity may be the most freezable fraction (Asahina 1966). And it has been assumed that the gut contents and haemolymph are the two most likely site of nucletion in insects (Salt 1964, 1966). Overwintering larvae of the giant bagworm moth cease feeding in the fall. From these point of view, it seems that the volume effect of supercooling can be shown when the water or liquid of biological materials is contained in capillary tubes such as the vascular tissues of conifer needles, but not in spaces such as the body cavity of insects.

Changes of mean supercooling points and percent body moisture from fall to spring

Changes in mean supercooling points, percent body moisture and minimum air temperatures from Nov. 1964 to Apr. 1965 are shown in Fig. 3. There were no marked seasonal changes in supercooling points of larvae of either sex and their difference between sexes continued throughout the winter. In general, supercooling points of univoltine insects exhibit periodicity, being high in summer, dropping in autumn, reaching a minimum during winter, and rising again in spring.

Percent body moisture of female larvae was higher than that of males by 1.7 % in November, but thereafter the relation reversed because the decrease of percent moisture in female larvae during winter exceeded that of males. This may probably be related to sexual differences of water loss (decrease of body weight) during winter as will be described later.

Table 1. Glycerol contents and fresh body weights of larvae in either sex.

Male larvae		Female larvae	
Fresh body weight (mg)	Glycerol (%)	Fresh body weight (mg)	Glycerol (%)
410	0.19	1304	0.26
395	0.10	1094	0.18
386	0.11	1034	0.17
340	0.18	921	0.22
296	0.18	794	0.23
Mean 0.15		Mean 0.21	

Presence of glycerol

The glycerol contents of five larvae of each sex in February were determined. Glycerol was found in both male and female larvae. As shown in Table 1, the content of glycerol was slightly higher in females than in males. Although sorbitol was also detected in both sexes, its content was not determined quantitatively.

Supercooling and survival at −13.5 C

The mean supercooling points of larvae in mid-winter were about −14 C in females and −18.5 C in males. Overwintering larvae were exposed to about the supercooling point, −13.5±1.0 C, of females for 44 hours, and their ability to supercool and survive was examined. The percentage unfrozen (supercooled) female larvae decreased as the exposure time lengthened, and all of the females froze and died within 44 hours, whereas about 80 % of males could supercool (Fig. 4). Thus, the sexual difference in supercooling and survival at −13.5 C agrees with the supercooling data described above. However, about half of the unfrozen male larvae died even in supercooling at −13.5 C for 44 hours.

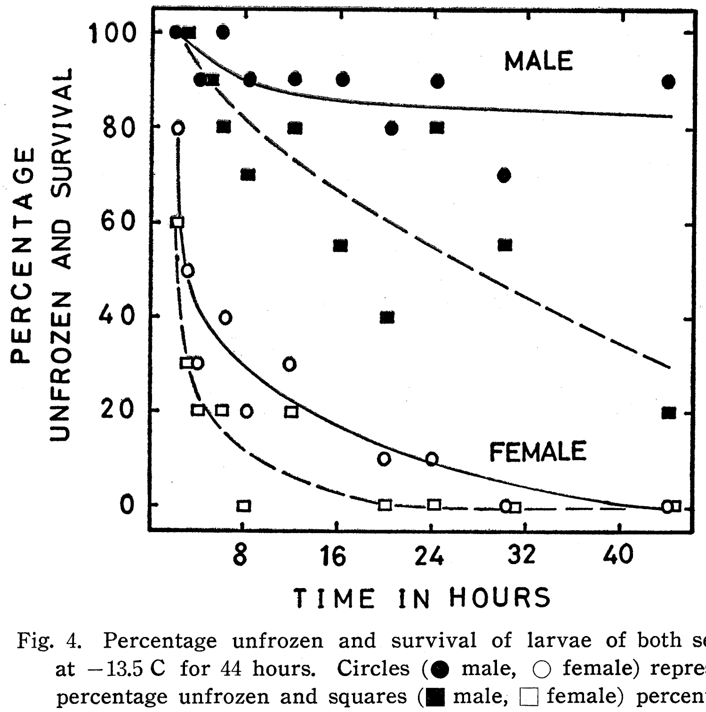

Fig. 4. Percentage unfrozen and survival of larvae of both sexes at −13.5 C for 44 hours. Circles (● male, ○ female) represent percentage unfrozen and squares (■ male, □ female) percentage survival. Each point means different set. Linnes fitted by eye.

Sexual differences of water loss and percent survival

Water loss (decrease of body weight) and percent survival were compared in both sexes, outdoors and at 2 C. As shown in Fig. 5, water loss in females was much larger than that of males at each cold treatment. That is, the body weights of males were decreased by about 20 % by March, compared to 40 to 50 % in females. From this result it is seen that the decreaee of percent body moisture of females exceeded that of males during winter (Fig. 3).

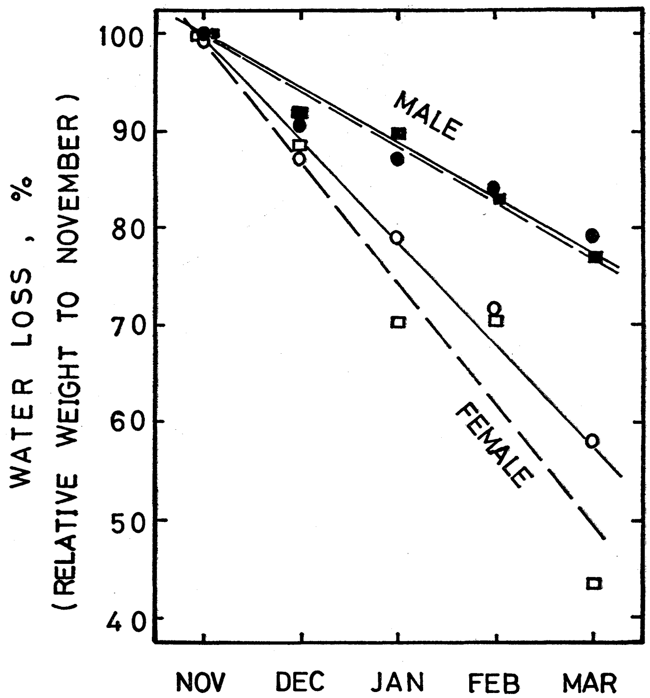

Fig. 5. Sexual differences of water loss outdoors in winter (solid line) and at constant cold, about 2 C (broken line). Lines fitted by eye.

In addition, percent survival in females was also lower than that of males and its decreasing tendency was more pronounced at the constant cold temperature than the outdoors (Fig. 6). The long constant cold exposure to 2 C as well as the outdoor conditions of winter in Fukuoka, affected the survival of larvae, especially females. Thus, it is probable that the larvae of giant bagworm moth are not only freezing-susceptible but also chilling-sensitive. Since the geographic distribution of this species is limited to south-western Japan and the minimum temperatures during winter in the area are approximately −10 C, it is apparent that the

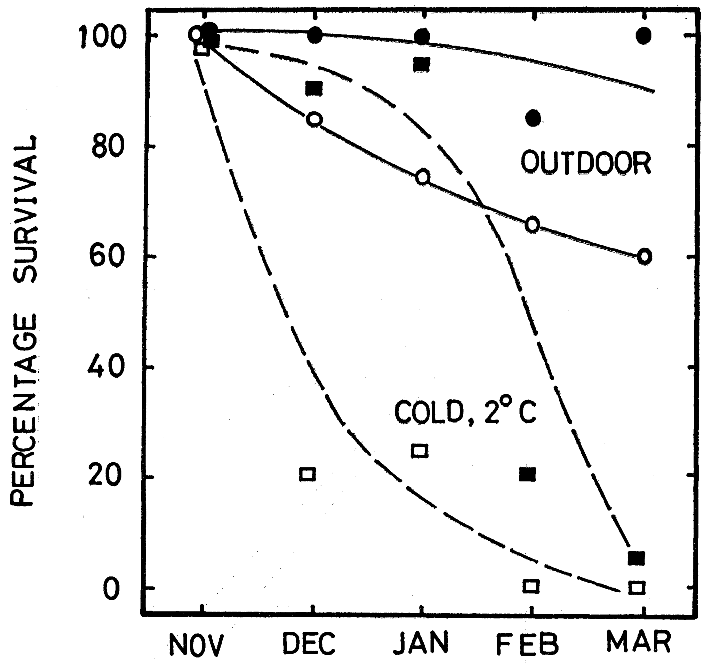

Fig. 6. Changes of percentage survival of larvae of both sexes outdoors in winter and at constant cold, about 2 C. Symbols as in Fig. 5. Lines fitted by eye.

supercooling ability of larvae is sufficient for avoidance of freezing in either sex. Thus, there must be another lethal factor in overwintering of this species. As seen in Fig. 5, water loss during winter is much larger in female larvae than that of male. This marked decrease of body weight in female larvae might probably be critical for their winter survival. Actually, we can often find many dehydrated female larvae in cocoons in spring. Water loss from fall to spring may be a critical factor in the survival of overwintering female larvae.

Summary

Sexual differences of supercooling and water loss in overwintering larvae of giant bagworm moth, *Clania variegata*, were found. Male larvae were smaller in size and weight than females and supercooling points of the former were about 4 C lower than those of the latter throughout the winter. Within the same sex, however, no size dependence of supercooling points could be detected.

Male larvae have larger supercooling and survival abilities than female larvae at −13.5 C. Regardless of sex, all of the larvae died after

freezing. Also, about half of the male larvae died while supercooling at −13.5 C for 44 hours, The mortality in female larvae was much larger than in males either outdoors during winter or at the constant cold about 2 C. This may probably related to the larger water loss in female larvae.

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