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Notes on the Larval Hibernation of *Epilachna admirabilis* (Coleoptera : Coccinellidae)[1,2]

Kazuo HOSHIKAWA[3]
星 川 和 夫

Abstract Hibernating habits, super-cooling points and frost resistance of larvae of the ladybird *Epilachna admirabilis* are described. Based upon its chorological records, possible factors limiting their northern distributional boundary are discussed.

Introduction

Most insects in the temperate to arctic regions hibernate at the species-specific developmental stage, which is more or less common among the species related at certain phyletic levels. This accordance suggests similar adaptive tendencies in hibernation. In Coccinellidae, too, their so far known hibernating stage is mostly the adults, with exception of only a few cases (5, 15). *Epilachna admirabilis* Crotch represents such exception, hibernating at the final instar larvae (prepupae) (7, 8, 9, 17). Further, it was recently observed that a fraction of adults pass again their second winter (9, 7). In other words, this species has two different hibernating stages ; one is the matured larvae which is normal for the species but exceptional for the family, and another is adults which is exceptional for the species but prevailing for the family. This situation may provide good opportunities to study how and why the hibernating stage is fixed through the adaptation to environmental conditions under which hibernation takes place.

However, the hibernating adults are scarce and difficult to obtain. In this preliminary report, therefore, the factors limiting their distribution are discussed in relation to super-cooling points and frost resistance of the larvae.

Before going further, I wish to express my sincere thanks to Prof. Shôichi F. Sakagami and Dr. Kôzô Tanno for their reading through the manuscript. Cordial thanks are also due to Prof. S. Takagi, Entomological Institute, Hokkaido Univ., and to Dr. H. Katakura, Zoological Institute, Hokkaido Univ., who kindly allowed me the examination of the specimens deposited in the collections under their care.

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2 Studies on the life cycle of *Epilachna admirabilis*. I

3 The Institute of Low Temperature Science, Hokkaido University, Sapporo, 060

Materials and Methods

The hibernating larvae were observed and collected from the northern slope of Mt. Maruyama, suburb of Sapporo City, northern Japan, in winter (Dec. 1, 13, 27, 1978; Jan. 12, 1979; and Jan. 12, Oct. 22, 29, Nov. 5, 12, 1980). For comparison, the prehibernating and feeding larvae collected on Sept. 28, 1979 were also examined.

Super-cooling points for both winter and autumn samples were measured using the method by Tanno (18), which consists of two alternative treatments: with and without artificial ice inoculation. Each larva was covered in triple with a small vinyl sack (ca. 10×15 mm), a glass vial ($3\phi \times 8$ cm) and a plastic vial ($6\phi \times 10$ cm), and put into a freezer. Thermal change was pursued by a thermocouple, the tip of which was lain on the venter of the larva. In case of artificial inoculation, the larva was wrapped with a piece of wet absorbent cotton or of wet dead leaf before measuring super-cooling point. The degree of frost resistance was evaluated by the ratio of individuals survived after measuring super-cooling point. The survival of the frozen larva was judged by both movement and color change 48 hours after being thawed.

Results and Discussion

Hibernating site of the matured larvae

The hibernating larvae were found under dead leaves in litter (102 examples) or on living leaves of a fern laying on the litter (15 ex.). Rarely they remained on the completely withered leaves of the host plants, *Schizopepon bryoniaefolius* Maxim. or *Gynostemma pentaphyllum* (Thunb.) Makino (2 ex.).

The litter, from which most hibernating larvae were collected, was more or less wet at the biginning of winter season (Nov. and Dec.), and repeated

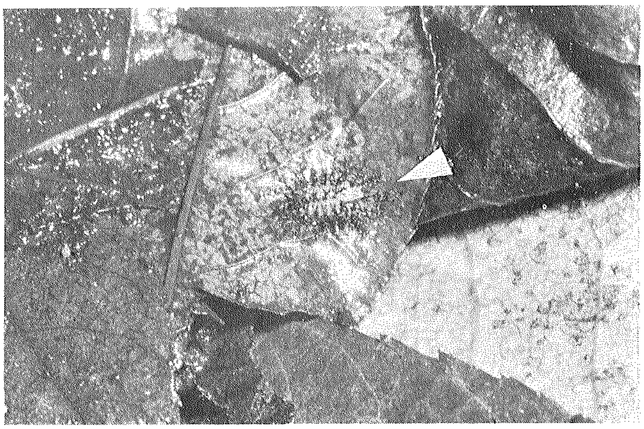

Fig. 1. The larva of *Epliachna admirabilis* hibernating under the surface of a dead leaf. Wet leaves are reflecting the light.

freezing and thawing. Once frozen, the litter was so firm as hard to strip off leaves, even under snow cover about 10 to 20 cm deep (Dec. 27 and Jan. 12). A maximum-minimum thermometer buried in the litter from Dec. 3, 1979 to Apr. 29, 1980, indicated −6.1°C in the minimum. These circumstancial evidences on the litter suggest that most hibernating larvae in this habitat could be inoculated by ice. No larvae had died when they were collected. Among 119 larvae collected from hibernating population, most were the 4th instar, but a 3rd instar one was found in the litter. Such immature larvae should die in the next spring for lack of food even if they succeeded hibernating.

Super-cooling points and frost resistance

In case of artificial ice inoculation, the water surrounding larva began to freeze (S_W) before the larva was frozen (S_L) (Fig. 2, A). Supper-cooling points obtained from these freezing curves are given in Fig. 3, left-hand. Since super-cooling points can change with cooling rate (1, 2), mean cooling rates between +2°C and −2°C are also given.

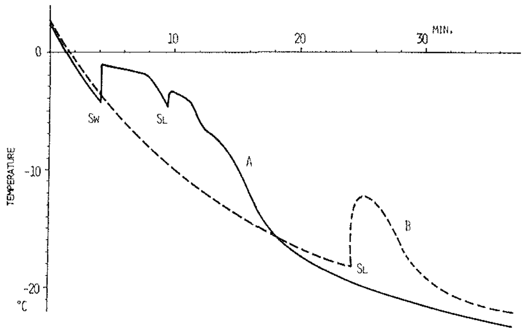

Fig. 2. Freezing curves of hibernating larvae of *E. admirabilis*. A : inoculated, B : spontaneous, S : super-cooling points (S_L : that of larva, S_W : that of water)

In autumn samples, the difference by the two treatments was less conspicuous : Larvae were frozen at −4.56°C (s. d.=±0.97 ; n=5) in case of inoculation, while at −7.38°C (±0.51 ; n=12) spontaneously. Food remaining in the digestive tube should act as inoculator in the latter case (14).

In winter samples, a marked difference in super-cooling points was found between inoculation (−5.29±1.87°C ; n=62) and spontaneous freezing (−18.62±3.01°C ; n=27). The former does not much differ from that in autumn samples, while the latter becomes much deeper. The difference is more obvious at slower cooling rates. This fact shows that inoculator in the larva was lost during October. However, any devices protecting larvae from inoculation by environmental ice could not be found. The hibernating larvae were easily frozen by inoculation, at least, under the experimental conditions.

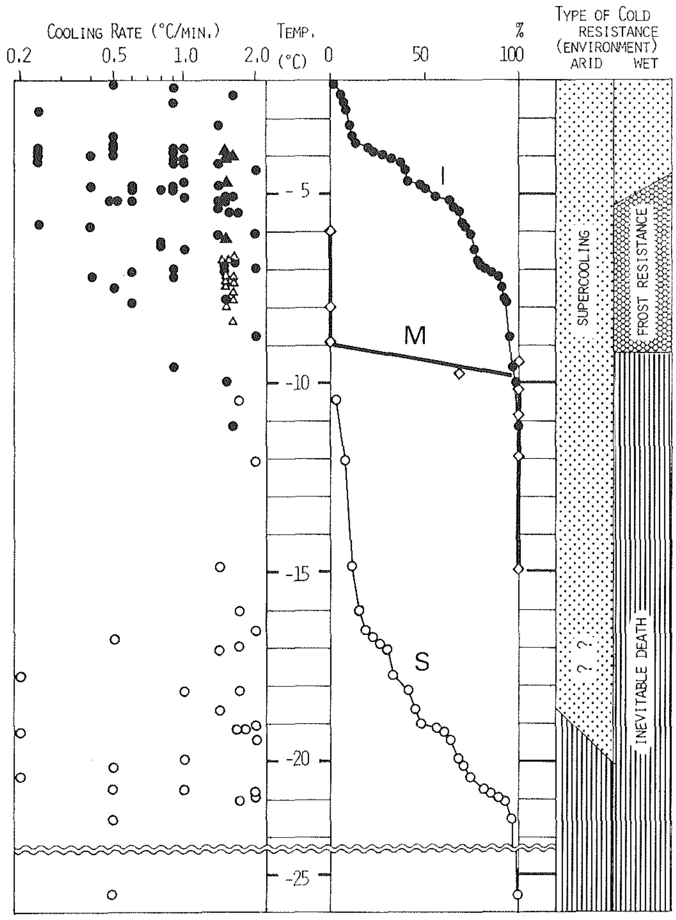

Fig. 3. Super-cooling points and frost resistance of hibernating larvae of *E. admirabilis*. Left : Super-cooling points by different cooling rates, circle: winter sample, triangle: autumn sample, closed: inoculated, open: spontaneous. Middle: Cumulative percentages of super-cooling points (I : inoculated, S : spontaneous) and mortalities when the larvae were kept at respective temperatures for 24 hours after freezing by inoculation (M). Right : Tolerant zones for coldness

Cold and frost resistance of the larvae were examined using 3~6 individuals for each temperature. In spontaneous freezing no larvae frozen could survive after thawing. Larvae could survive, however, when they kept in super-cooling condition, at least, −11.5°C for 24 hours. In freezing by ice inoculation, on the other hand, all larvae died after frozen and kept below −10°C for 24 hours. When kept above −9°C for the same duration, on the contrary, all larvae were observed to move after thawing. Eleven out of 12 individuals frozen at −12°C with ice inoculation for a short time (within 15 min.) were observed to move after thawing. This might be explained by that the amount of ice in the frozen insect body did not reach the equilibrium at the temperature (1). But such short duration freezing should not occur under natural condition. Thus, the hibernating larva is of the maximum ability in its frost resistance for 24 hours about $-9.5°C$, when the slow freezing is undertaken by the ice inoculating technique.

Distribution of the species

Based on the bibliographical survey (3, 7, 8, 9, 10, 11, 15, 16, 17) and examination of the deposited specimens, a tentative distribution map was prepared (Fig. 4). Within the available information, the northern boundary of *Epilachna admirabilis* in Japan lies the area near Sapporo, where the present study was carried out. The northernmost records are those from Ikushunbetsu and Tôbetsu chô, both in Hokkaido.

Their main host plants in Hokkaido, *Schizopepon bryoniaefolius* and *Gynostemma pentaphyllum*, certainly do not delimit the northern boundary, as they are distributed throughout Hokkaido (4, 13). On the other hand, the degree of frost resistance by inoculation treatment (about $-9.5°C$) is not so differ from the minimum environmental temperature in Sapporo (about $-6°C$). The both temperatures are nearly the same, taken into consideration annual fluctuation in the environmental one. For comparison,

Fig. 4. Distribution of *E. admirabilis*. A: The specific grobal distribution, B: Collection records in Japan, C: Isotherms of the normal monthly mean of daily minimum air temperature in December (6). Area hatched: below $-10°C$

some isotherms of the normal monthly mean of daily minimum air temperature in December (6) are shown in Fig. 4, C. The ladybird had never been collected from the area where the normal mean temperature below −10°C.

This leads to an unusual conclusion. The northern boundary of this species just attains its physiological limit for frost resistance. Such would be quite rare in other species, because many insects may stop the northward colonization before attaining their physiological limit, by intervention of various ecological factors. Anyhow, further studies are required to clarify the problem especially on the following items, i) whether and to what extent the hibernating larvae are actually frozen by inoculation in the field, ii) whether they can avoid such wet environment, particularly in their northern territory, by selecting more favorable sites for hibernation or not, iii) how high is winter mortality in actual hibernating sites of diverse conditions.

Setting aside these questions, the present case should be regarded as disharmony between physiological tolerance for coldness and ecological attributes, showing a contrast with the case of *Sciara* sp., in which the hibernating larvae in wet dead stems of their host plant show fair frost resistance when they are frozen by ice inoculation (18).

It is also noteworthy that *Epilachna admirabilis* was collected from Okushiri island. Since the island have not been connected with Hokkaido during the ice ages (12), the species should have been able to tolerate the coldness there in the ice ages, unless the postglacial colonization is assumed.

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