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Notes on Adult Hibernation of *Epilachna admirabilis*, with Special Reference to Comparison with Larval Hibernation (Coleoptera: Coccinellidae)[1,2]

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Abstract. *Epilachna admirabilis* hibernates by two life stages, mature larvae and adults, of which ratio fluctuates annually. Their hibernating habits are described with some features of the hibernating site, litter layer. In spite of being a temperate species, this species can tolerate freezing in both larvae (about −9.5°C for 24 hrs) and adults (−7.5°C) when frozen by artificial ice inoculation. Except for trehalose (2.5∼4.0% to body weight) neither sugar nor sugar-alcohol is accumulated in the hibernating individuals. These ecological and physiological attributes related with hibernation are compared between adults and larvae.

Introduction

Some arctic and alpine insects require more than one year in completing their life cycle, during which they hibernate twice or more. In such case the two or more hibernating stages in the same species are same or different, e. g. in the alpine butterflies in Mt. Daisetsu, *Oeneis daisetsuzana* hibernates by larvae, either young or mature, while *Parnassius eversmanni* does by eggs and pupae (16). For the lack of a sufficient growing season, they should have adopted such life cycle strategy. In the process of this adaptation, they had to acquire the ability to tolerate the coldness in severe winter there in more than one stage. Recently the frost resistance in both larvae and adults of the arctic beetle *Pytho americanus* was confirmed (15). An incipient state of such ability might be expected also in temperate species.

Epilachna admirabilis Crotch is distributed in the warm-temperate region of East Asia, and has a puzzling life cycle at their northernmost range, hibernating not only by mature 4th instar larvae but partially and occasionally also by adults (12, 8, 5). In the present paper several ecological and physiological attributes of the two hibernating stages are compared, as a basis to solve the problem how their life cycle is regulated.

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

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Materials and Methods

The hibernating population on the northern slope of Mt. Maruyama, suburb of Sapporo City, northern Japan, has been observed since 1978. Unless mentioned, the following descriptions are based on the weekly or monthly survey during 1980–'81. Environmental temperature of the hibernating site was persued by a platinum thermistor connected with a cordless recorder (Mitogiken Ltd.) and a maximum-minimum thermometer. The minimum air temperature was measured simultaneously by a minimum thermometer hung under a tumbled tree 1.2 m above the ground. All samples for dissection, experiments and chemical analysis were obtained from the same population. Twelve females were dissected in 0.85% NaCl solution to examine their ovarian conditions and the possession or not of spermatozoa.

Super-cooling points, both by spontaneous freezing and by ice ionculation as well as the degree of frost resistance were measured by the methods reported previously (5). Sugar and sugar-alcohol contents were analyzed by a gas-liquid chromatograph, of which detailed procedures are described elsewhere (6). Other details on methods are given below at appropriate pages.

Results and Discussion

I. *Annual fluctuation of hibernating stages.*

As mentioned above this species hibernates normally by mature larvae, while occasionally and partially by adults. The ratio of the two hibernating stages has fluctuated from winter to winter. In 1978～'79 and '79～'80, 96 and 23 hibernating larvae were collected respectively, but no hibernating adults were obtained. In winter 1980～'81, however, 109 females and 185 males of hibernating adults were collected together with 83 larvae.

The hibernating larvae had issued from the eggs laid in the prior

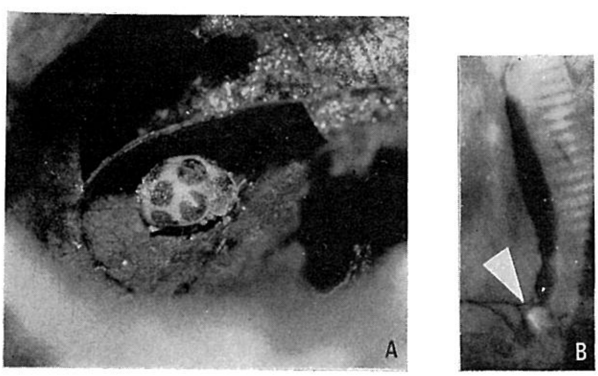

Fig. 1. **A**: The adult *Epilachna admirabilis* hibernating under the surface of a dead leaf. Water droplets on the elytra shows the moist environmental condition. **B**: An ovariole of hibernating female, with corpus luteum (reversed tone)

summer. They seem to enter diapause under a particular photoperiod condition in the autumn at the area studied (7). On the other hand, the hibernating adults should not have emerged in the prior autumn but have entered in their second winter. Each ovariole of the hibernating females had an orange-colored patch, corpus luteum (Fig. 1, B), which indicated a previous ovarian development (11). All 7 examined females contained spermatozoa in their "sperm node" (cf. 9).

The hibernation of this Maruyama population was incidentally recorded twice in the past: "Most of newly emerged adults seemed to hibernate" and "a small number of adults and a large number of 4th instar larvae were found in litter" (12), and "approximate ratio of individuals hibernated by adults to the whole hibernated individuals falls ca. 8%" based on seasonal fluctuation of adult numbers counted during May to October in 1975 (8). Thus, the ratio adults/(adults+larvae) in the hibernating population fluctuated as follows:

1951～'52:	less than half.
1974～'75:	ca. 8%.
1978～'79:	0% (0～4% by 95% confidence limits).
1979～'80:	0% (0～15%).
1980～'81:	78% (73～82%).

The causes of this curious fluctuation are now being studied.

II. *Ecological observations.*

The adults were found in their hibernating site from early October to mid May, whereas the larvae arrived there in mid and late October, and left there in mid May. On May 10, 1981, some adults and larvae had left the litter, but seed leaves of the host plant *Schizopepon bryoniaefolius* were not yet fed. The earliest feeding by posthibernating adults was observed on May 22 on both normal and seed leaves. At that time, the other host plant of this population, *Gynostemma pentaphyllum*, was about to bud, on which no feeding was observed. The latter was fed from early June. Although most posthibernating larvae pupated without feeding, a few larvae fed on a small amount of *S. bryoniaefolius*. On October 5, 27 out of 28 larvae were found on host plants, while 1 larva and 11 adults were already in litter or on leaves of ferns. No adults were observed on host plants in October.

The hibernating sites of adults and larvae were the same, i. e. litter layer surrounding their host plants. No concentration of hibernating insects in particular depth was observed within the litter layer ca. 2～8 cm in thickness. Temperature in the litter layer was nearly constant (near 0°C) when covered with snow more than 30 cm deep, while daily fluctuation was considerable when the litter was exposed, especially in spring (Fig. 2). The minimum temperature (−3.0°C) was recorded on December 18. These

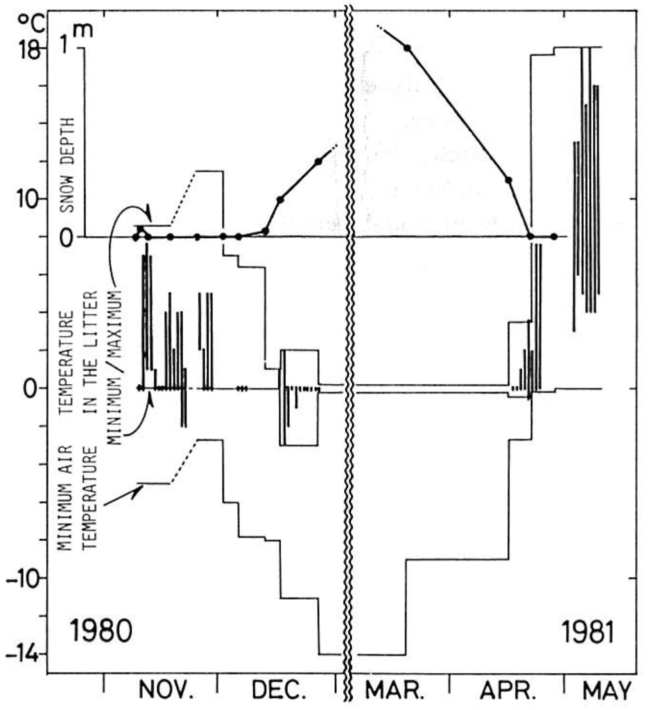

Fig. 2. Seasonal change of temperature and snow depth in the hibernating site, litter layer, Maruyama, Sapporo, in 1980–1981. Ranges of daily fluctuation are given by vertical columns.

tendencies are similar to the case in a dead stem of *Lilium cordatum* at 10 cm above the ground used as hibernaculum by *Sciara* sp. (18). Ranges of fluctuation were, however, fairly narrow in the present case. A large amount of water contained in the litter should act as a thermal buffer, particularly at sub-zero zones. The amount of water contained in the litter was measured by the difference between original wet weight (WW) and dried weight (DW). The ratio WW/DW was 2.6 on Nov. 25 and 3.9 on Dec. 6, indicating a fairly soaked condition (about 10～20 g of water in 100 cm^2 of the litter).

Densities of the hibernating adults and larvae in the litter were 7.7 adults/m^2 (s. d.=2.7, $n=6$) and 1.7 larvae/m^2 (s. d.=1.2, $n=6$). Although precise quantitative data were unavailable, the larval density in 1980～'81 seemed to be lower than in 1978～'79 or '79～'80.

Mortality during hibernation was estimated by the ratio of dead individuals found in a sample collected at the end of hibernation. Overestimation might be caused by counting individuals which had died before hibernation and by disturbance due to trampling at census, and underestimation, though less probable, by ignoring the effect of predators who devour each insect entirely. Eleven out of 112 adults and 4 of 29 larvae had been died when collected on Apr. 29 (9.8% and 13.8%, respectively, without significant difference at 95% confidence). However, at least in this winter, the posthibernating larvae should hardly have contributed to maintain the population, as 33 out of 37 reared larvae (89%) had been infested by the monoparasitic wasp *Disoymus afissae* Watanabe. Kurosawa, who first found this parasite, also observed 80% of hibernating larvae had been infested in 1951~'52 (12). The higher ratio of parasitized larvae might be a result rather than a cause of lower density of the larvae.

Hibernating site choice test: To make the preference for hibernating site of diverse conditions clear, a simple choice test was carried out (Fig. 3). Dead leaves, which had once been dried and mixed with various amounts of water, were put in a plastic box (24×12×8 cm). Marked 18 individuals were released at boundaries of masses of dead leaf of different water contents. The box was first kept at 10°C, under which the insects can freely move, for 1 hr (for adults) or 3 hrs (for larvae), then at 5°C, under which they usually do not move, for 1 hr. Direction and distance of movement were measured for each individual. The test was repeated twice for each stage. Although the moisture gradient became loose during the test, most of adults and larvae moved to relatively arid sections. This shows that they prefer rather arid place when hibernating, neverthless, they were found actually in extremely wet litter (Fig. 1, A). There was no arid litter in the hibernating site in November and December under natural conditions. A large scale prehibernating migration should not be made by this species.

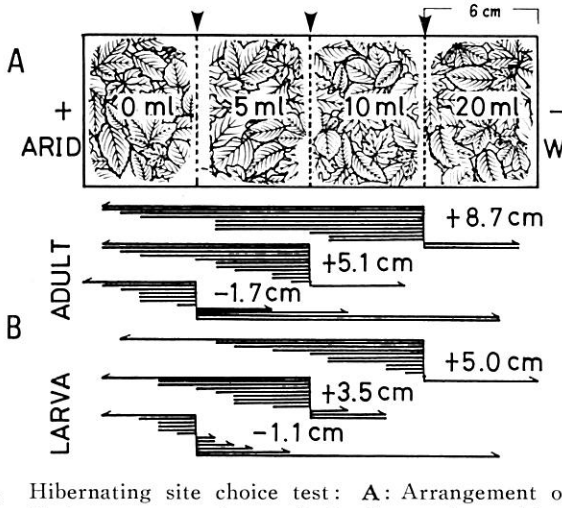

Fig. 3. Hibernating site choice test: **A**: Arrangement of the test. Various amounts of water (0–20 ml) were thoroughly mixed with a mass of dried dead leaves (8 g) and put in a box. Insects were released at the points marked with triangles, before cooling the box: room temp.→release, 10°C (1 hr for adults, 3 hrs for larvae)→5°C (1 hr). **B**: Direction and distance passed by each insect during the test, with mean distance moved from each releasing point

III. *Physiological tolerance for the coldness.*

Super-cooling points of the hibernating adults were measured with and without ice inoculation (Fig. 4). Like in the larvae (5), the adults were easily frozen by ice inoculation at −4.15°C on the average (s. d.=1.15, n=67; Fig. 4, I), suggesting lack of protective devices from inoculation by environmental ice.

In spontaneous freezing, on the other hand, the mean super-cooling point was −13.51°C (s. d.=4.33, n=47; Fig. 4, S). However, the mean value shifted to −15.38°C (s. d.=3.22, n=22; Fig. 4, S′) when measured subsequently with the same specimens which had once frozen and died, though the difference was not significant at 95% confidence limits. The discrepancy

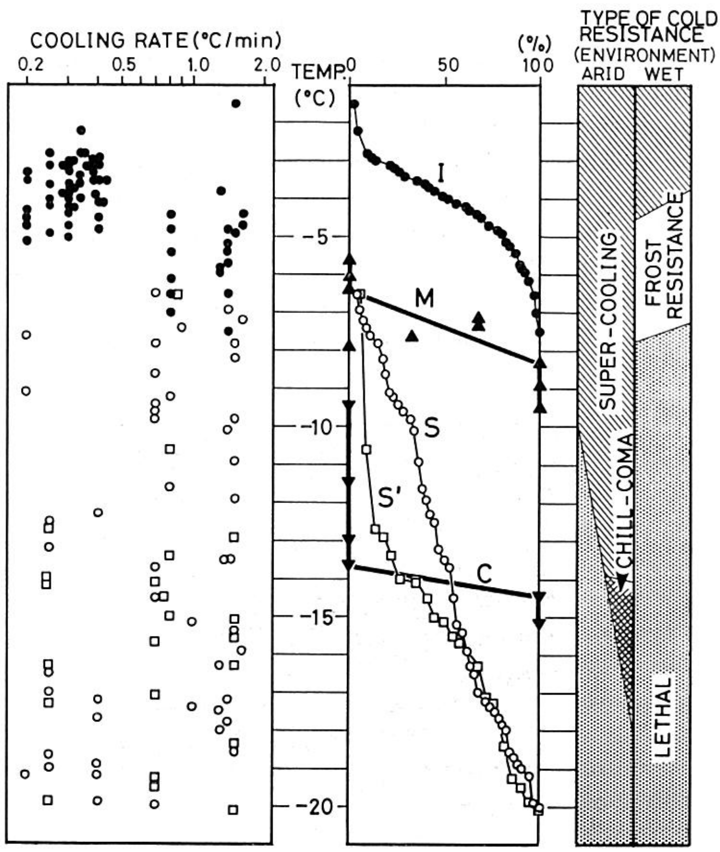

Fig. 4. Super-cooling points, frost resistance and chill-coma induction in hibernating adults of *Epilachna admirabilis*. **Left**: Super-cooling points by different cooling rates. Open circle, spontaneous (the first trials); open square, do (the second trials); closed circle, inoculated. **Middle**: Cumulative percentage of super-cooling points. **I**, inoculated; **S**, spontaneous (the first); **S′**, do. (the second). **M**, Mortality after freezing by inoculation; **C**, ratio of individuals fallen into chill-coma, both in adults kept at respective temperatures for 24 hrs. **Right**: Tolerant zones for coldness

could be interpreted by i) the first higher values include artifacts produced when the ladybird had been wrapped, since they are apt to bleed even when slightly touched. The exuded fluid may act as an inoculator. Or, ii) water loss during measurement may lower the super-cooling point. Since super-cooling is a stochastic phenomenon, if the latter interpretation is valid, we can expect a linear regression of the difference between the first and second super-cooling points (Y) to the first value (X), as $Y_i = X_i + 15.38$. On the other hand if the former interpretation is valid, Y should be more than the expected value only in the specimens frozen at higher temperatures in the first trial. In fact, calculated regression line was $Y = +0.84\ X + 13.27$ without larger deviations in the specimens with high first super-cooling point. This favors the latter interpretation and the adults are regarded as frozen spontaneously at about −13.5°C.

By spontaneously freezing all adults and larvae have died. Some individuals avoided freezing when they had been exposed for 24 hrs at a temperature at which the spontaneous freezing could occur. But even in these, their behaviour after rewarming was abnormal. By chill-coma* their legs were cramped, making normal movements impossible, only trembling of antenae and mouth parts. These conditions were observed in adults at −14.5°C (2 ex.) and −15.2°C (5 ex.) and in larvae at −17.3°C (2 ex.) and −17.7°C (2 ex.). There was no visible effect in the adult exposed at −9.5°C (6 ex.), −11.5°C (3 ex.) and −13.0°C (5 ex.), and also in the larvae exposed at −11.5°C (3 ex.), −13.7°C (2 ex.) and −16.0°C (3 ex.) (Fig. 4, C, for adults). Although adults exposed at −13.7°C (2 ex.) were scarcely able to walk just after rewarming to ca. +10°C by loss of leg co-ordination, they recovered normally 8 days after. Thus, the chill-coma affected irreversibly below −14°C for adults and −16.5°C for larvae. Ecologically, the irrecoverable chill-coma is identical with the death.

Frost resistance was observed only in freezing by inoculation, using 4∼6 adults for each temperature for 24 hrs (Fig. 4, M). Adults frozen by inoculation could survive at −5.4°C, −6.0°C and −6.4°C but not at −8.3°C, −8.9°C and −9.5°C. Mortality fluctuated at temperatures between −7 and −8°C. Thus, the maximum frost resistance for 24 hrs of the hibernating adults was about −7.5°C when frozen by inoculation.

The hibernating larvae of this species also exhibit a frost tolerance (5). This is a second case of the insect which tolerates freezing in more than one life stage, following the first record in *Pytho americanus*, of which larvae and adults can tolerate freezing at −40°C and −45°C respectively (15). Despite a large difference in the degree of frost resistance, super-cooling of these two species are similar from each other: *Epilachna* larvae (−5.3°C), adults (−4.2), *Pytho* larvae (−4.1 to −7.1), adults (−4 to −8.7).

* Here the chill-coma is defined as inability to walk normally, irrespective of the condition is irrecoverable or not.

But there is a crucial difference: *Pytho* should have nucleating agents which ensure ice formation in their haemolymph (15). On the contrary, *Epilachna* should not have such special nucleating agent in their body and are frozen by external ice inoculator. This difference reflects, together with that in frost resistance, their provenances: a species in the warm temperate region and an arctic endemic.

IV. *Sugars in the hibernating insect.*

The sugars, mainly trehalose, began to be accumulated before November, and rapidly decreased in May (Table 1). Although the entire seasonal variation of sugar contents has not been clarified yet, the larvae may accu-

Table 1. Sugar contents in hibernating larvae and adults of *Epilachna admirabilis*, shown with percentage to fresh body weight. TRE: trehalose, MS: monosaccharides (probably glucose), DS: disaccharides (probably sucrose)

Date of collection	Larva				Adult				
	TRE	MS	DS	Total	TRE	MS	DS	Total	Sex
Nov. 5*					0.60	0.52	0.09	1.22	♀
					0.72	0.18	0.05	0.95	♂
					0.88	0.30	0.02	1.21	♂
					1.10	0.15	0.05	1.31	♀
Nov. 13	1.33	0.12	0.08	1.52	0.99	0.02	0.02	1.03	♂
	2.17	0.02	0.22	2.39	1.19	0.06	0.04	1.29	♂
					1.39	0.02	0.05	1.47	♀
Nov. 23	2.79	0.10	0.11	3.01	1.52	0.12	0.04	1.68	♂
	3.01	0.05	0.10	3.16	1.84	0.08	0.03	1.94	♀
	3.37	0.19	0.19	3.77	1.88	0.09	0.02	2.00	♂
Dec. 4	2.81	0.04	0.18	3.04	1.92	0.06	0.10	2.08	♂
	3.00	0.13	0.11	3.24					
Dec. 11	3.66	0.08	0.16	3.90	2.03	0.04	0.06	2.13	♀
	3.67	0.20	0.07	3.94	2.12	0.05	0.06	2.24	♂
	4.07	0.04	0.18	4.29	2.20	0.04	0.06	2.31	♀
					2.57	0.06	0.12	2.75	♀
					2.66	0.07	0.12	2.85	♂
Mar. 30	2.33	0.04	0.06	2.43	2.11	0.18	0.01	2.31	♂
	2.56	0.31	0.02	2.90	2.30	0.09	0.02	2.42	♀
					2.37	0.18	0.06	2.61	♀
					2.83	0.09	0.02	2.94	♂
Apr. 10	2.49	0.08	0.08	2.66	2.60	0.08	0.11	2.80	♂
May 2	2.18	0.04	0.12	2.35	0.70	0.01	0.47	1.24	♂
May 9					0.76	0.07	0.03	0.90	♂
May 12	0.18	0.07	0.10	0.36	0.40	0.06	0.03	0.50	♂
May 27	0.29	0.06	0.04	0.40					
Jul. 21					0.01	0.13	0.02	0.16	♂
					0.02	0.27	0.01	0.33	♂

* In 1980, others in 1981

mulate or decrease trehalose rapidly than the adults, of which maximum content is presumably about 4% in larvae and 2.5~3% in adults. A small amount of monosaccharides (probably glucose) and disaccharides (probably sucrose) also appeared in gas-liquid chromatograms. No other sugars and sugar-alcohols were found except some traces. Trehalose was found in prepupae of the popular sawfly *Trichiocampus populi* (7.1%) (17), and adults of the carabid beetle *Pterostichus brevicornis* (3.6%), in the latter accompanied with much glycerol (11.8%) (2). These species can tolerate freezing even at −40°C, while *E. admirabilis* only above −9.5°C or −7.5°C. Correspondingly, the amounts of these substances are lesser in this species.

In adults collected in December and kept in a refrigerator (ca. 5°C) for 100 days, trehalose content was only 0.71% of the fresh body weight (s. d. = 0.08, $n=5$), despite the individuals from natural population held 2.5% at that time. The difference should relate to the temperature they had experienced, ca. 5°C vs. 0°C. The trehalose synthesis in a gall fly is accelerated by low temperature (14). Such slight difference in temperature at appropriate time might bring a notable effect for hibernating insects.

V. *Comparison between adult and larval hibernation of* Epilachna admirabilis

Notwithstanding adults and larvae of this species are quite different in the morpho-functional make-up, their ecological and physiological attributes related with hibernation are similar from each other (Table 2). Differences

Table 2. Synopsis of the attributes concerning larval and adult hibernation of *Epilachna admirabilis* in Sapporo

	Hibernating stage	
	Larva	Adult
Ecological		
Beginning of hibernation	mid/late Oct.	Sept.
End of hibernation	—— mid May ——	
Hibernating site	—— litter layer ——	
Potential preference in choice of hibernating site	—— arid ——	
Mortality during hibernation	ca. 14% =	ca. 10%
Physiological		
Super-cooling point		
by spontaneous freezing	−18.6°C	−13.5°C
by inoculation	− 5.3°C	− 4.2°C
Frost resistance		
by spontaneous freezing	—— absent ——	
by inoculation	above − 9.5°C	above − 7.5°C
Temperature inducing irrecoverable chill-coma	below −16.5°C	below −14.0°C
Amount of trehalose accumulated	ca. 4%	ca. 2.5–3%

are : i) the adults enter hibernation earlier than the larvae, ii) all temperatures related with cold resistance ; super-cooling points, lower limit in frost resistance and upper limit of chill-coma induction, are lower in larvae than in adults and iii) trehalose content is higher in larvae. Summarizing, the larval attributes are more favorable for cold resistance even though slightly. As far as the present study goes, hibernation of adults and larvae is similar in three aspects : i) Both are protected by snow cover during most of winter season. ii) Both are protected from rapid temperature change by ample moisture in the hibernating site. iii) Both tolerate freezing caused by inoculation with environmental ice before snowing to some degree. Thus, the adults and larvae adopt quite similar hibernating strategies, though physiologically the larvae are more suitable.

Pytho americanus, another species proven to be frost tolerant in two life stages, also exhibits similar trends between adults and larvae (15). In case of *Epilachna* and *Pytho*, the same strategy adopted by two different stages may be either manifestation of the species-specific potential for cold tolerance, which can appear in any stage adopted for hibernation, or a mere parallelism caused by similar adaptations to the same hibernating site. These two assumptions are not exclusive but further comparative studies are required to ascertain their relative validity.

Concerning the relative antiquity, the larval hibernation should be ancestral in *Epilachna admirabilis* by the reason given below, despite this is unique in Coccinellidae, in which adult hibernation prevails. Suppose the following combinations between ancestral hibernating stage and physiological suitability for hibernation of a species :

	physiological suitability		
ancestral hibernation	adult $\gg$ larva /	adult = larva /	adult $\ll$ larva
larval (L)	L^-	L^o	L^+
adult (A)	A^+	A^o	A^-

In the course of the northward distribution of a species, no change of hibernating stage is expected in L^+ and A^+, whereas such change can occur in L^- ($L \rightarrow A$) or A^- ($A \rightarrow L$). This reasoning should be valid also in case L^o or A^o, which approximately corresponds to the present case. *Epilachna admirabilis* is an essentially warm temperate species (cf. 5 for global distribution map*). Unfortunately, hibernation in southern populations is poorly known except fragmentary records of larval hibernation in Tokyo, Honshu (10) and probably in China (13). Adult hibernation have not been observed there. This suggests that larval hibernation monopolizes or at least prevails in the southern range, therefore, the antiquity of larval hibernation ($\fallingdotseq L^0$).

* In a recent monograph of Chinese ladybirds (19), the following localities of the species are unmentioned though these were noted previously (13): Peking City, Anhwei Province, Kiangsi Prov., and Hupeh Prov.

Then, adult hibernation may be of a recent acquisition in *Epilachna admirabilis*. Annual fluctuation of adult hibernation favors this opinion. The adult hibernation might have appeared by a potential derived from the ancestral Epilachninae species, in which this hibernation type is exclusive as in most coccinelids. Such phyletic restriction is also found in some Japanese alpine butterflies hibernating twice (5 spp.). Most of them hibernate by species-specific stages prevailing in the family to which they belong (cf. 16). In the course of adaptation to the temperate winter each species should have chosen independently their hibernating stage within the fundamental design of their life cycle. If the species acquired an enough cold tolerance in more than one stage, and if other eco-physiological conditions permit its survival in the northern areas, it may invade and establish there, lengthening their life cycle involving more than one hibernation. Practical significance of the adult hibernation for the Maruyama population may be the maintenance of their population density.

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