Kontyû, Tokyo, **50** (4): 599–603. December 25, 1982

Long Mating Season and its Bearing on the Reproductive Isolation in a Pair of Sympatric Phytophagous Ladybirds (Coleoptera, Coccinellidae)*

Haruo KATAKURA

Zoological Institute, Faculty of Science, Hokkaido University,
Sapporo, Hokkaido, 060 Japan

Synopsis The seasonal changes in the frequency of inseminated females of *Henosepilachna pustulosa* and *H. vigintioctomaculata* in and near Sapporo, northern Japan, were studied. Although the females of these univoltine beetles emerging in midsummer do not lay eggs until the next spring, more than half of them are inseminated before entering hibernation. The implication of this finding is discussed in relation to the reproductive isolation between these two closely related species.

Henosepilachna vigintioctomaculata complex is a series of closely related phytophagous ladybirds and both morphologically and biologically very diversified (KATAKURA, 1981a). Two or three species of this complex are sympatric in various parts of Japan. *H. pustulosa* (KÔNO) and *H. vigintioctomaculata* (MOTSCHULSKY) are one of such sympatric pairs. The reproductive isolating mechanisms between these beetles have been studied (KATAKURA, 1976, 1981a; KATAKURA & NAKANO, 1979; NAKANO, 1980, 1981). So far as known, they are mainly isolated by the host plant difference and the low hatchability of eggs produced by interbreeding. Some other factors, i.e., the mating preference, multiple mating and phenological difference, further strengthen their reproductive isolation.

Both *H. pustulosa* and *H. vigintioctomaculata* are univoltine and hibernate as adults (KATAKURA, 1976). Adults appear on the host plants in spring, feed for a while and begin to lay eggs. The oviposition period lasts about a month or more. Mating is frequently observed in these pre-oviposition and oviposition periods. New adults emerge in midsummer, feed on the main and some subsidiary food plants, and enter hibernation in fall without laying eggs. However, they mate occasionally before hibernation. To assess the efficiency of such mating activities, females of these beetles were periodically sampled in 1981 at several localities in the vicinity of Sapporo, Hokkaido, northern Japan, and their sperm storage organs were examined. Based on the result obtained, I report evidence for the long-continued mating activity of these beetles intervened by hibernation, and discuss its bearing on the reproductive isolation between them.

* Contributions to the knowledge of *Henosepilachna vigintioctomaculata* complex. XII.

Materials and Method

Beetles examined in the present study were the form P-III or Sapporo form of *H. pustulosa* and the form V-II or Hokkaido form of *H. vigintioctomaculata* (KATAKURA, 1974). Regular sampling was made at four localities for *H. pustulosa* (both before and after hibernation: Maruyama, Tsukisamu; only after hibernation: Kobetsuzawa, Nopporo) and three for *H. vigintioctomaculata* (before and after hibernation: Tsukisamu, Kitahiroshima; after hibernation: Todoyama). Additional sampling was made at some other localities (cf. Fig. 1). Most individuals of *H. pustulosa* were collected from thistles (*Cirsium* spp., Compositae) or blue cohosh (*Caulophyllum robustum* MAXIM., Berberidaceae), the two main host plants of this species in and near Sapporo (KATAKURA *et al.*, 1977). Some were collected from potato (*Solanum tuberosum* L., Solanaceae) and other crop plants. Most adults of *H. vigintioctomaculata* were collected from potato or, in fall, from various crop plants and weeds near potato fields. At Kobetsuzawa, they were caught on *Schizopepon bryoniaefolius* Maxim. (Cucurbitaceae), a probable native host plant of this species in Hokkaido (KATAKURA, 1975, 1981a). The collected beetles were dissected in RINGER's solution and their sperm storage organs (KATAKURA, 1981b) were examined. A few old adults of *H. pustulosa* collected in midsummer to fall with new adults were distinguished by their degenerated ovaries and omitted from the results given below.

Results

The results are summarized in Fig. 1. After hibernation adults of *H. pustulosa* began to appear on the host plants in early May, 1981, while *H. vigintioctomaculata* in early June. This difference was probably related to the phenological difference of the respective host plants (cf. KATAKURA, 1976). In both species, a considerable proportion of those adults were already inseminated. This was the case even in the earlier period of their appearance on the host plants. For example, 64 (71.9%) of the 89 individuals of *H. pustulosa* examined in the earlier half of May were inseminated. Likewise, 71 (83.5%) of the 85 females of *H. vigintioctomaculata* collected in the earlier half of June were inseminated.

The first samplings of new generation adults were made when most of them had emerged. Some of them had already mated. The proportion of inseminated females increased towards the fall and about half of the beetles collected in September were inseminated. Frequency of inseminated females in the newly emerged ones was 52.6% (40/76) in *H. pustulosa* and 40.6% (56/138) in *H. vigintioctomaculata*. Since new generation adults were observed till late September or early October after the last samples were collected in mid September, the proportion of inseminated females in the hibernating populations might be higher than that shown in Fig. 1. Sperm activity was examined for about half of the beetles collected both before and

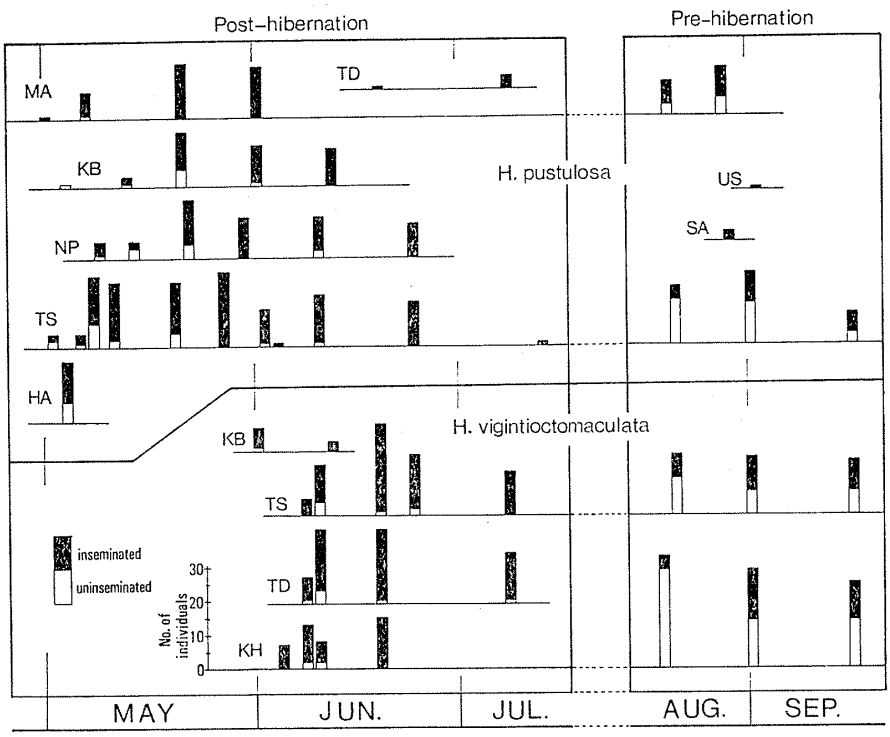

Fig. 1. Seasonal change in the frequency of inseminated females of *H. pustulosa* and *H. vigintioctomaculata* at several localities in and near Sapporo, northern Japan, in 1981. Abbreviations of localities: MA, Maruyama; TD, Todoyama; KB, Kobetsuzawa; NP, Nopporo; TS, Tsukisamu; HA, Hariusu; US, Usubetsu; SA, Sankakuyama; KH, Kitahiroshima.

after hibernation. Without exception, spermatozoa were alive and active when the sperm storage organ was broken in RINGER's solution.

Discussion

As mentioned above, more than half of the newly emerged females of *H. pustulosa* and *H. vigintioctomaculata* seem to mate before entering hibernation. This was unexpected since mating by the new adults was only occasionally found in the field. Mating prior to hibernation is rather common in other coccinellid beetles (HODEK, 1973; HOWARD & ENGLISH, 1924; TANIGISHI, 1976), and may be significant in their reproductive biology. Although it is yet unknown whether spermatozoa received by the females of *H. pustulosa* or *H. vigintioctomaculata* before hibernation are kept viable for a long period of time, at least part of them may be capable of fertilizing eggs in the next spring. The high frequency of inseminated females immediately after the resumption of spring activity (Fig. 1) and the fact that they can lay fertilized eggs for even one month or more after the last mating in the laboratory (NAKANO, pers. comm.) support this assumption. The

mating season of these beetles is thus very long, from the summer of emergence (August) to the end of oviposition (early July of the next year) with the intervention of winter dormancy. Oviposition occurs from late May (*H. pustulosa*) or mid June (*H. vigintioctomaculata*) to early July (cf. KATAKURA, 1976).

H. pustulosa and *H. vigintioctomaculata* are generally segregated from each other by their different preference for host plants. However, they are often found together on some kinds of plants due to the overlapping range of their food plants (KATAKURA *et al.*, 1977; KATAKURA, 1981a). Partial sexual isolation between these beetles was observed in the laboratory (KATAKURA & NAKANO, 1979), but interspecific matings are not rare in mixed populations (NAKANO, 1980). Assuming that pre-hibernation mating would not be effective for the reproduction in the next year, I previously inferred that the coexistence of new adults of *H. pustulosa* and *H. vigintioctomaculata* would not lead to hybridization. This interpretation is invalidated by the present study. The results clearly show that there are chances of hybridization not only after but also before hibernation. The most crucial post-mating isolating factor between *H. pustulosa* and *H. vigintioctomaculata* is the low hatchability of eggs produced by the interspecific mating. Under the laboratory conditions, the hatchability of eggs produced by the interspecific mating is only 4% while that by the conspecific mating is 70% or more (KATAKURA & NAKANO, 1979). However, females of these beetles mate many times, and the deleterious effect of the interspecific mating is virtually eliminated by the previous or later conspecific mating (NAKANO, 1980). Hatching ratio does not significantly drop by an interspecific mating if the female has already mated with a conspecific male, or the low hatching ratio caused by an interspecific mating drastically rises close to the normal level after a conspecific mating. Further, almost all offspring of such females are similar to their mothers (NAKANO, 1980). Under such circumstances, it is advantageous for the females in mixed populations to mate many times. The more frequently a female mates, the more likely she mates with a conspecific male. Probability of making conspecific mating is increased by the presence of sexual, though weak, isolation between *H. pustulosa* and *H. vigintioctomaculata*. The long mating season of these beetles reported in this paper contributes to the reinforcement of their reproductive isolation in mixed populations by increasing mating frequency and thus the chance of conspecific mating.

Acknowledgement I wish to express my thanks to an anonymous reviewer who read and criticized an earlier draft of the manuscript.

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