Entomologia Experimentalis et Applicata **75:** 145–149, 1995.
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Synchronous growth of a parasitoid, *Perilitus coccinellae*, and teratocytes with the development of the host, *Coccinella septempunctata*

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Accepted: September 8, 1994

Key words: host-parasitoid relationship, teratocyte, diapause, methoprene, *Perilitus coccinellae*, *Coccinella septempunctata*

Abstract

The relationship between the development of *Coccinella septempunctata brucki* Mulsant (Coleoptera : Coccinellidae) and its parasitoid, *Perilitus coccinellae* (Schrank) (Hymenoptera : Braconidae) was studied at two photoperiods (L16:D8 and L12:D12) at 26 °C. The development of *P. coccinellae* is well synchronized with the physiological state of the host, *C. septempunctata*, which can be parasitized not only as adult but also as larva or pupa. The parasitoid larva completed larval development within 19 days in a non-diapausing host, while in diapausing adults as well as in pupae held at diapause-averting conditions, the parasitoid larva ceased growth at the first instar. Growth was resumed when diapause of the host terminated or by the emergence of the adult host from the pupa.

About 550 spheric cells, teratocytes, were liberated into the host hemocoel when the parasitoid egg hatched. The teratocytes increased in size in the active host, while their development was arrested in the diapausing host. Application of methoprene caused diapause termination of both host and parasitoid larva. The results indicate that the development of the larva of *P. coccinellae* depends on the physiological conditions of the host, *C. septempunctata brucki*. The host-parasite relation thus represents an 'endogenous synchronization' in the sense of Schoonhoven's definition.

Introduction

The braconid wasp *Perilitus coccinellae* (Schrank) is a parthenogenetic solitary endoparasitoid of lady beetles. About 30 species of aphidophagous lady beetles are recorded as natural hosts of the wasp (Hodek, 1973). The life history of these host coccinellids differs from species to species (interspecies variability) and even from place to place (intraspecies variability). Thus, it is of interest to examine how the life cycle of *P. coccinellae* is tuned to diverse life cycles of the hosts.

The seven spotted lady beetle *Coccinella septempunctata brucki* Mulsant is a host of *P. coccinellae* in Japan (Maeta, 1969). The coccinellid has been reported as bivoltine in central Japan. The first generation enters imaginal diapause in the summer while the adults of the second generation hibernate (Sakurai *et al.*, 1987). The aestivation diapause is controled by photoperiod and temperature, i.e., long day and high temperature enhance the diapause incidence (Okuda & Hodek, 1983).

It has been reported in some hymenopteran parasitoids (Braconidae, Trichogrammatidae, and Scelionidae) that a number of spheric cells are liberated into the host hemocoel after hatching of parasitoid larvae. Several investigations have shown that the cells, named teratocytes, originate from serosa (Ogloblin, 1924; Jackson, 1935; Lawrence, 1990). Although several possible functions of the teratocytes have been suggested (Dahlman, 1990), concrete evidence is still scarce. In this paper we study the development of both the

larvae of *P. coccinellae* and the teratocytes in relation to different physiological states of *C. septempunctata*. The probable function of teratocytes is discussed.

Materials and methods

Insects. Parasitized beetles, *C. septempunctata*, were collected from the field in Gifu prefecture, central Japan. In this area the parasitization rate by *P. coccinellae* ranges between 7 to 45% (Kadono-Okuda, unpubl. data). The emerged parasitoid wasps were fed on 30% sucrose solution until their use in experiments. The host beetles were reared under laboratory conditions at 26 °C. Non-diapause and diapause beetles were obtained by rearing them under short day (L12:D12) and long day (L16:D8) conditions, respectively (Okuda & Hodek, 1983; Sakurai *et al.*, 1987). Coccinellids were fed on aphids (mainly *Acyrthosiphon pisum*).

Parasitization. A parasitoid wasp was placed together with four or five host beetles/larvae in a petri dish (4.5 cm diameter) and allowed to parasitize the coccinellids. After it was stung, each beetle/larva was immediately removed individually from the petri dish to avoid superparasitization.

Methoprene application. Five μg of methoprene was dissolved in 5 μl peanut oil and was topically applied to a diapause host beetle on the 16th day after parasitization.

Results

Development of parasitoids in non-diapausing and diapausing adults. The development of egg and pupa of *P. coccinellae* in the host *C. septempunctata* in different physiological states was determined by daily dissection of the parasitized hosts. When a non-diapausing adult host was parasitized at the age of 14 days, it took on average 17 days from egg to emergence from the host (Fig. 1-A). The daily increase of the parasitoid larva in size is shown in Fig. 2. The body length increased gradually up to 9 days after parasitization and just after molting into the second instar, the increase in body length was more rapid. The increase in body width was similar (data not shown). Last molting to the third instar occurred just prior to emergence from the host.

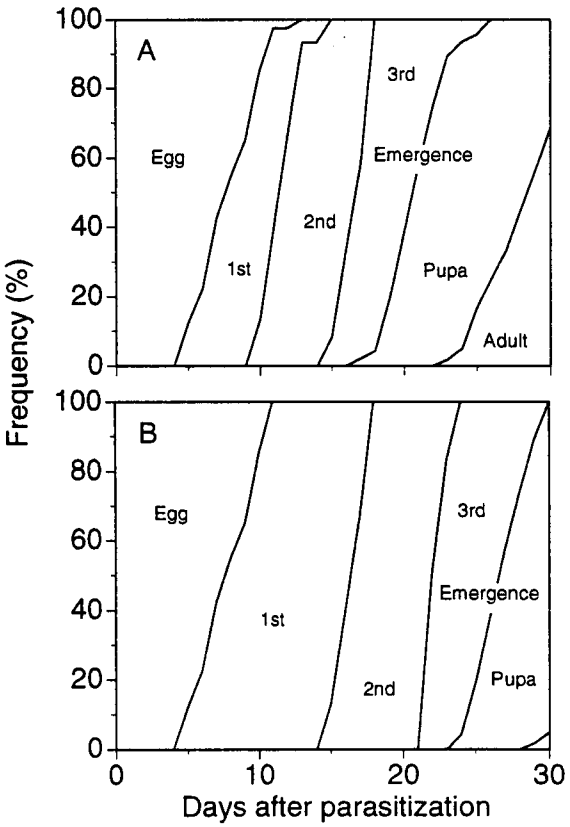

Fig. 1. Pre-pupal development of parasitoid within the host at the different stages. A: adult hosts were parasitized at day 14 after emergence. B: larval hosts were parasitized in the last instar. Numbers represent the larval instar of the parasitoid. Dissection was carried out every day after parasitization. Five to eight parasitized hosts were used in each sample.

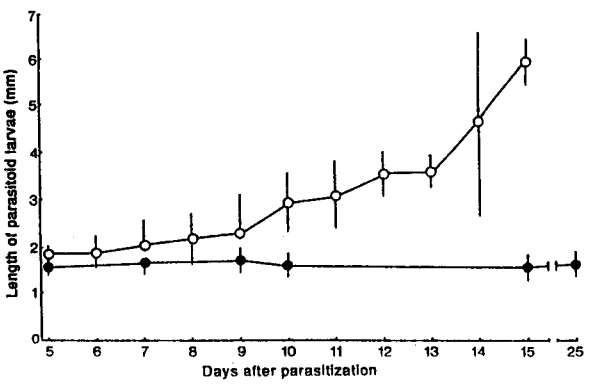

Fig. 2. Changes of parasitoid larva in size within the adult host at different physiological stages. Open circles: hosts, reared under diapause preventing conditions, were parasitized at day 14 after emergence. Closed circles: hosts reared under diapause promoting conditions were parasitized at day 14 after emergence. Five to eight parasitized hosts were used in each sample (mean±SD).

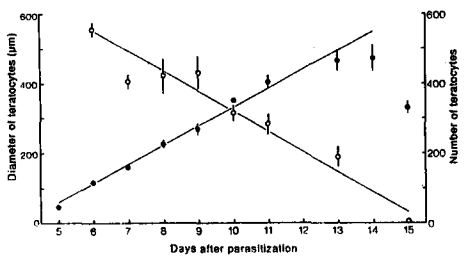

Fig. 3. Changes of teratocytes in size and number within non-diapausing adult hosts. Five to eight parasitized hosts were used in each sample. Open circles: mean number of teratocytes. Closed circles: mean diameter of teratocytes. Vertical lines represent SD.

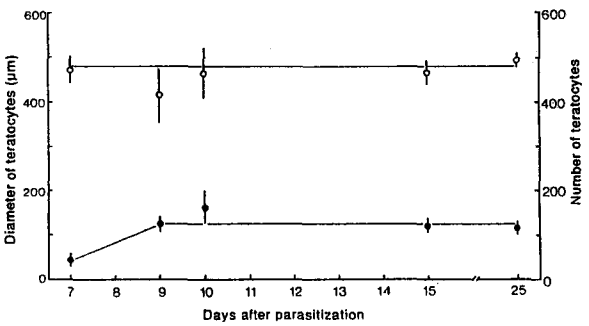

Fig. 4. Changes of teratocytes in size and number within diapausing hosts. Five to eight parasitized hosts were used in each sample. Open circles: mean number of teratocytes. Closed circles: mean diameter of teratocytes. Vertical lines represent SD.

When a diapausing host was parasitized at the age of 14 days, the parasitoid larva ceased its development at the first instar (Fig. 2). The parasitoid diapause lasted until the termination of host diapause.

Development of parasitoids in larval hosts. P. coccinellae usually parasitizes adult coccinellids. But when only coccinellid larvae are present, the wasps eventually parasitize the larvae. In case of parasitization of the last larval instar (Fig. 1-B), emergence of the parasitoid larva from the host took place 22 days after parasitization, which was about 5 days longer than the development in an adult non-diapausing host. The arrest of larval development of the parasitoid occurred at the 1st instar. The resumption of the development of the parasitoid larvae coincided with the adult emergence of the host beetle.

Teratocyte growth in non-diapausing and diapausing hosts. The daily changes of size and number of teratocytes within active host beetles are shown in Fig. 3. Just after hatching of the parasitoid larva, teratocytes were in a clump, so that it was difficult to count their number precisely. The size of the teratocytes was approximately 47 μm in diameter. Later, teratocytes dispersed in the host hemocoel and on the 6th day after parasitization the number amounted to about 550. On the 14th day after parasitization teratocytes reached their maximum diameter, about 500 μm. During parasitization, the number of teratocytes decreased to such an extent that either a few or no teratocytes were observed in the body cavity of the host just before or after emergence from the host.

Both the parasitoid development and the teratocyte growth were arrested in a diapausing host. The diameter of teratocytes remained at about 100 to 150μm throughout the host diapause (Fig. 4) and their development was resumed only with the termination of the host diapause.

Effect of JH analogue treatment on the development of parasitoid larvae and teratocytes. The host beetles reared under diapause inducing conditions were parasitized on the 14th day after adult emergence. On the 16th day after parasitization, 5 μg of methoprene was topically applied to the parasitized host beetles. The methoprene treatment induced vitellin accumulation in the host ovaries. Later, however, vitellin resorption took place in the ovaries (data not shown) due to resumption of development of both the parasitoid larvae and the teratocytes after the methoprene treatment (Figs. 5, 6). In non-treated hosts all the parasitoid larvae remained in diapause.

Discussion

The rate of larval development of *P. coccinellae* differed according to the physiological state of the host. Parasitoid larvae in diapausing hosts stopped growing and remained at the 1st instar until the diapause of the host was terminated (Fig. 2), while the pre-pupal development in non-diapausing hosts was completed within an average of 19 days (Figs. 1, 2). Synchronization of the life cycle of the parasitoid with its host could be established by two mechanisms (Schoonhoven, 1963): (a)'endogenous synchronization', i.e., stimulation or inhibition of the development of parasitoid by its host; (b) 'coincidence', i.e., synchronization of the two life cycles by external stimuli. The relationship between

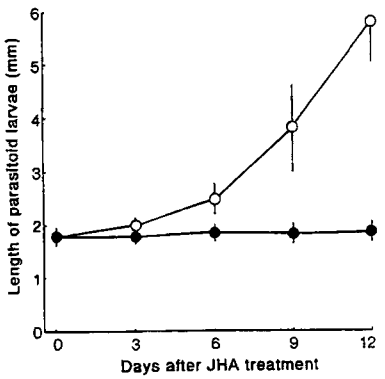

Fig. 5. Changes of parasitoid larva in size after methoprene treatment. Hosts reared under diapause promoting conditions were parasitized at day 14 after emergence. Five μg methoprene was applied topically to the parasitized host at day 30 after host emergence. Open circles: methoprene treatment. Closed circles: control. Five parasitized hosts were used in each sample (mean±SD).

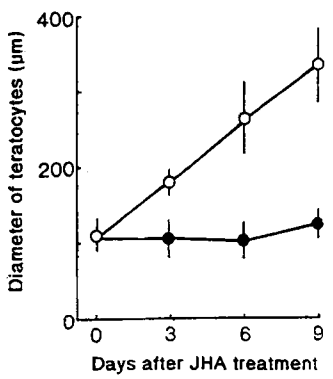

Fig. 6. Changes of teratocytes in size after methoprene treatment. Conditions are the same as in Fig. 5. Open circles: methoprene treatment. Closed circles: control. Five parasitized hosts were used in each sample (mean±SD).

P. coccinellae and its host, *Coleomegilla maculata* has been categorized as (b) 'coincidence', because the host and parasitoid responded independently to photoperiod during maintenance and termination of diapause (Obrycki & Tauber, 1978).

Our results, however, indicate that the relationship between *P. coccinellae* and *C. septempunctata brucki* can be categorized as (a)'endogenous synchronization', because the parasitoid development seemed to be more affected by the host's physiological condition than by external stimuli such as photoperiod. The first instar parasitoid larvae ceased growing in the host at the pupal stage under diapause averting conditions (Fig. 1-B), while the parasitoid larvae did not exhibit any arrest in the adult hosts under the same rearing conditions. This indicates that first instar *P. coccinellae* larvae wait for suitable physiological conditions of the host for further development. Teratocytes were well synchronized with parasitoid development (Fig. 4). The parasitoid larva has less chance to influence the teratocytes than the host, indicating that growth of both parasitoid larva and teratocytes are dependent on the host's physiological condition. Treatment with a JH analogue, methoprene, induced diapause termination of both the parasitoid and its host (Fig. 5). It is unlikely that larval diapause is terminated by the direct effect of the methoprene, because larval diapause is induced and maintained by the application of JH (Yagi & Fukaya, 1974; Yin & Chippendale, 1976). This result is thus another indication of the 'endogenous synchronization'.

In two experiments conducted in France, one result indicated a direct dependence of the development of *P. coccinellae* on the physiological conditions of *C. septempunctata septempunctata*, while the other suggested also the involvement of environmental conditions (Hodek *et al.*, 1978). Thus we concluded that the host dependence or independence of prepupal development of *P. coccinellae* differed according to the host species. This dependence of response on the host species has already been reported in *Apanteles glomeratus* (Maslennikova in Tauber *et al.*, 1983); when *A. glomeratus* parasitizes *Pieris brassicae*, its diapause response is largely independent from that of its host, but when *A. glomeratus* parasitized *Aporia crataegi*, its diapause is determined by the physiology of its host.

Teratocyte number either remains the same (Tawfik, 1961; Hashimoto & Kitano, 1971; Vinson & Lewis, 1973) or decreases (Spencer, 1926; Jackson, 1935; Sluss, 1968) during parasitism. The decrease of teratocytes in number is thought to be due to feeding by parasitoid larvae (Ogloblin, 1924; Sluss, 1968; Arakawa & Kitano, 1989). Present results indicate that in *P. coccinellae* the teratocytes seem to have a nutritive role for parasitoid larvae because the number of teratocytes gradually decreased with growth of the parasitoid larvae and in the last stage the teratocytes disappeared completely from the host cavity (Fig. 3). Moreover, proteins extracted from the midgut of *P. coccinellae* parasitoid larva have shown an immunological reaction to the antibody against the teratocyte crude extract, indicating the ingestion of teratocytes by the parasitoid larva (Okuda and Kadono-Okuda, unpubl. data). In order to support this hypothesis, the characterization of teratocyte proteins is now being studied.

Acknowledgments

This work was partially supported by a national project 'Integrated research program on the development of insect technology'.

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