

Dynamics of Fat Content during Induction and Termination of “Trophic Diapause” in *Harmonia sedecimnotata* Fabr. Females (Coleoptera, Coccinellidae)

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Abstract—Experimental studies have demonstrated that onset, termination, and resumption of oviposition, same as corresponding changes in germarium size, are closely correlated with the dynamics of fat reserves. Even short-term (10 days long) termination of oviposition caused by the absence of aphid preys with the availability of carbohydrate food is accompanied by significant increase in absolute (weight) and relative (the percentage of total dry weight) fat content. This confirms the correctness of considering the studied phenomenon as a “real” reproductive diapause but not as a “short-term interruption of oviposition.” In combination with earlier published data, the results of the study once again suggest that division of insect adaptations to environmental changes into “short-term” and “long-term” adaptations based, correspondingly, on “neural” and “neurohormonal” mechanisms is rather conventional. In reality, a gradient or a spectrum of reactions is observed.

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It is well known that females of numerous carnivorous coccinellids deprived of aphid preys voluntarily fed on carbohydrate food (honey, sugar solution, etc.). In these cases, oviposition is terminated but it could be resumed when the possibility of feeding on aphids was provided. Studies conducted with various Coccinellidae species suggest that this phenomenon could be considered as a special form of reproductive (adult) diapause (Hodek, 1973; Zaslavskiy *et al.*, 1998).

The specificity of the object of our study, a tropical lady beetle *Harmonia sedecimnotata* Fabr. (Coleoptera, Coccinellidae) is that the absence of aphid food is a single environmental factor inducing termination of oviposition. Photoperiodic induction of diapause was not revealed in this species, although it is quite typical of insects. In addition, oviposition could be resumed surprisingly soon, 3–5 days after the first feeding on aphids (Zaslavskiy *et al.*, 1998; Semyanov, 2000; Semyanov, Vaghina, 1999, 2001).

This extraordinary ability for rapid termination and resumption of egg laying became the base for the hypothesis that this “trophic diapause,” in contrast to “photoperiodic diapause,” is nothing but a simple “interruption” of oviposition (Semyanov, 2002). Identity of mechanisms underlying “usual” and “trophic” diapauses in coccinellids has earlier been questioned by several other authors, who considered the phenomenon

under study as “a state analogous to the natural dormancy” (Iperti and Hodek, 1974), “diapause-like arrest of reproductive activity” (Hodek, 1983), etc. At the same time, the ability for fast resumption of oviposition *per se* was considered as a reason for using the term “quiescence,” but not “diapause” (Hagen, 1962).

It is well known, however, that termination of reproduction is the main but not the only feature of adult diapause. Reproductive diapause is usually accompanied by fat accumulation, by increased resistance to environmental extremes, and by other physiological and behavioral changes (Danilevskiy, 1961; Tauber *et al.*, 1986; Danks, 1987). The present research was aimed at quantitative analysis of dynamics of fat reserves during induction and termination of the “trophic reproductive diapause” in *H. sedecimnotata*.

MATERIALS AND METHODS

In our experiments, we used the laboratory strain of *H. sedecimnotata*, originated from insects collected in 1990 in China by V.P. Semyanov (Semyanov, 2000). The main methods of rearing and experimentation were the same as in the previous studies conducted with this lady beetle species (Zaslavskiy *et al.*, 1998; Semyanov, Vaghina, 1999, 2001, 2003).

Larvae and adults were fed with the green peach aphid, *Myzus persicae* Sulz., reared under laboratory

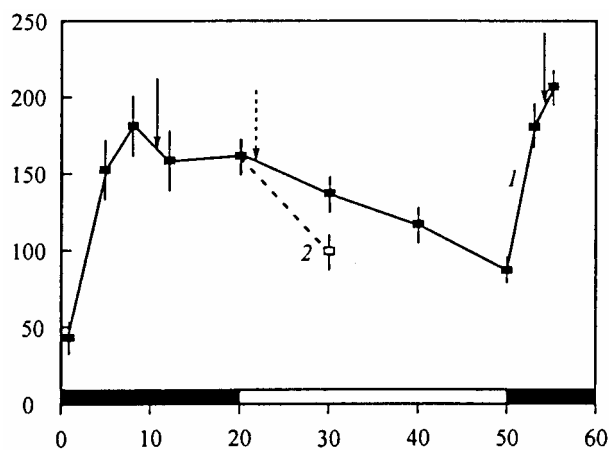


Fig. 1. The dynamics of hermarium size during females maturation, induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the first experiment). Abscissa: time (days); ordinate: hermarium size (multiplication of the length by the maximum width, mk). Hereafter, means and SD are shown. Diet: aphids (dark rectangles) or sugar solution (empty rectangles) is indicated above abscissa. Numbers indicate treatments; arrows indicate the mean time of onset (solid line) and termination (dotted line) of oviposition.

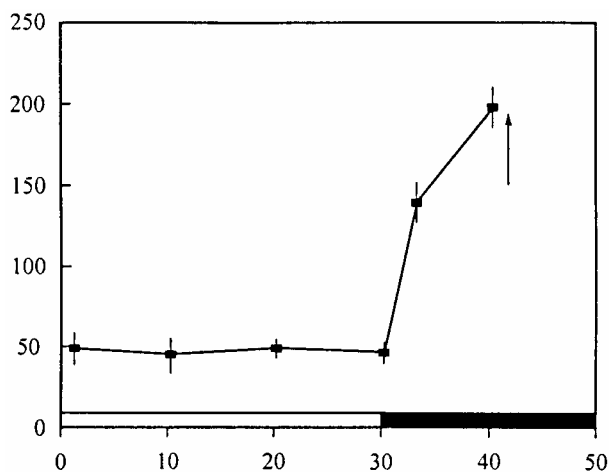


Fig. 2. Dynamics of hermarium size during induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the second experiment). For designations, see Fig. 1.

conditions on broad bean, *Vicia faba* L. plants. Sugar (10% water solution) was used as alternative carbohydrate food. All the experiments were conducted at a constant temperature of 25°C and under permanent light.

Fat content in females was estimated by commonly used (e.g., Ellers and van Alphen, 2002) extraction method. For each record, at any given day, 10 females were selected from the test group, killed with ether, dried in thermostatic chamber during 48 h at 40°C, and then kept in Petri dishes in a freezer (-18°C). The

beetles were weighed using electronic analytical balance "Ohaus AR 1530" within the accuracy of 0.001 g. To avoid water condensation, insects before weighing were gradually heated to room temperature inside the dishes. After weighing, the beetles were individually placed in jars with 20 ml of acetone and kept during three days at 20°C for extraction of fat. Next, the beetles were again dried under the same conditions and weighed. Thus, for each female its total dry weight (the first weighing) and weight of fat (difference between the first and the second weighings) were estimated. Basing on these two parameters, the relative fat content (percentage) was calculated.

In addition, certain females (not less than seven females for each record of each replicate of each treatment of each experiment) were dissected to estimate the intensity of the initial stages of oogenesis. For that, germarium length and maximum width were measured with a binocular microscope (3.2×10). Germarium size was approximated by multiplication of its length by the maximum width.

In both treatments of the first experiment (Fig. 1) emerged *H. sedecimnotata* females were offered aphid food during 20 days. Then in the first treatment they were fed on sugar solution. In the second treatment, the females, after 20 days of feeding on aphids, were provided only with pure water. In the first treatment, females after 30 days of feeding on sugar solution, were again allowed to feed on aphids. In the second treatment, practically all females, deprived both of aphids and of carbohydrate food have died till the 40th day of the experiment. In total, the first experiment lasted 55 days (Fig. 1). For germarium measuring and fat content estimation, random samples were selected from newly emerged females, on the 5th, the 8th, the 12th, and the 20th day of feeding on aphids, and on the 10th, the 20th, and the 30th days of absence of aphid food (correspondingly, 30, 40, and 50 days after the beginning of the experiment), and on the 3rd and the 5th day after the resumption of feeding on aphids (correspondingly, 53 and 55 days after the beginning of the experiment).

In the second experiment (Fig. 2), females were fed on sugar solution from adult emergence. Aphid food was provided only 30 days after the beginning of the experiment. Fat content was estimated on the 10th, 20th, and 30th days of feeding on sugar and on the 3rd, 5th, and 10th days of feeding on aphids (correspondingly, 33, 35, and 40 days after the beginning of the experiment). Hermariums were measured on the

same days (excluding the 35th day after the beginning of the experiment).

Two replicates of each experiment were conducted. Data of these replicates were similar and therefore they were pooled for the final treatment of the results. The Tukey test was used for paired comparisons of the data obtained on different days of the same experiment. To normalize the variation, percentages were arcsine-transformed ($Y = \text{Arc sin } \sqrt{x/100}$) before statistical treatment. All calculations were made using SYSTAT 3.0.

RESULTS

Hermarium Size

In the first experiment, when females were fed on aphids since emergence, hermarium size rapidly increased during the first 8–10 days of their life (Fig. 1). On the 12th day, when oviposition was initiated, slight but significant ($p < 0.01$, Tukey test) decrease in hermarium size was observed and then the size of hermarium was relatively stable. After the onset of feeding on sugar (the 20th day of the experiment), hermarium size gradually decreased. In the second treatment, when sugar solution was replaced with water, this decrease was sharply accelerated. Resumption of feeding on aphids (the 50th day of the first experiment) caused a rapid increase in hermarium size.

In the second experiment (Fig. 2) when the females were fed on sugar solution since emergence, hermarium size was almost constant during 30 days (the variations were insignificant). When aphid food was provided, hermarium size rapidly increased, and the pattern of this increase was similar to that observed in the first experiment during the maturation of young females and the reactivation of diapausing individuals (comp. Figs. 1 and 2).

Female Weight and Fat Content

When females were fed on aphids since emergence, their weight first increased and then gradually leveled off. The difference between mean weights of females recorded on 8th and on 20th days of their life was insignificant (Fig. 3). Feeding on sugar solution resulted in an increase of the weight of females (the difference between the means recorded on 20th and 50th days was significant at the $p < 0.001$ level), while resumption of feeding on aphids caused a sharp decrease in the mean weight of females (Fig. 3, 1). When

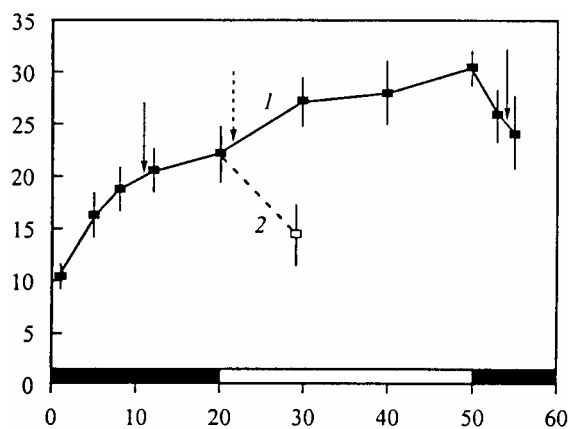


Fig. 3. Dynamics of dry weight during induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the first experiment). Ordinate: dry weight (mg). For other designations, see Fig. 1.

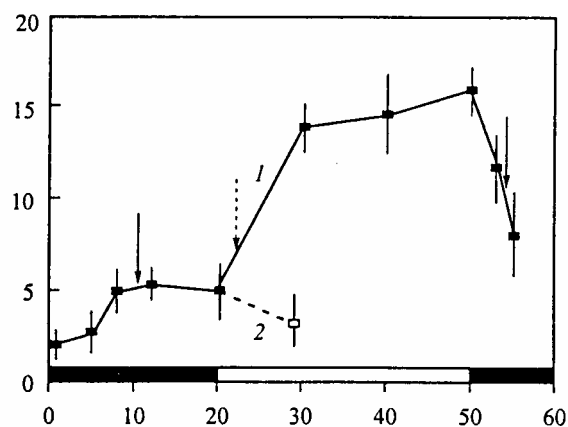


Fig. 4. Dynamics of fat weight during induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the first experiment). Ordinate: fat weight (mg). For other designations, see Fig. 1.

the females were provided with pure water instead of sugar solution, their weight decreased (Fig. 3, 2).

The dynamics of the absolute fat content was similar to that of the total dry weight of the female (comp. Figs. 3 and 4). When the females were fed on aphids since emergence, fat accumulation proceeded only during the first 5–8 days (Fig. 4). Feeding on sugar solution resulted in fast accumulation of fat and then (from the 30th to the 50th day of the experiment) variations of fat content were insignificant. Resumption of feeding on aphids induced a sharp decrease in fat content (Fig. 4, 1). Absence of carbohydrate food, as might be expected, prevented the fat accumulation (Fig. 4, 2).

Analysis of the dynamics of the proportion of fat in total dry weight of the female made it possible to refine the above picture (Fig. 5). When the females were

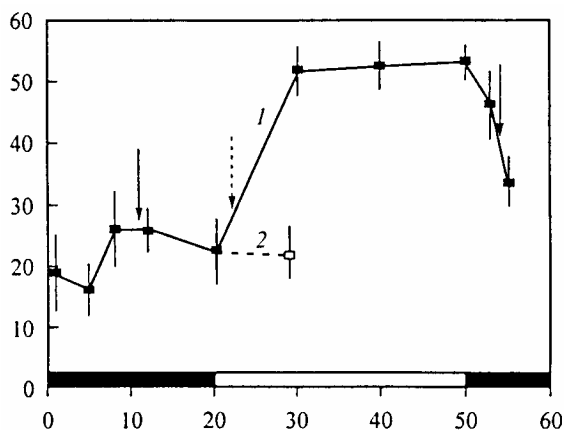


Fig. 5. Dynamics of fat content during induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the first experiment). Ordinate: fat content (%). For other designations, see Fig. 1.

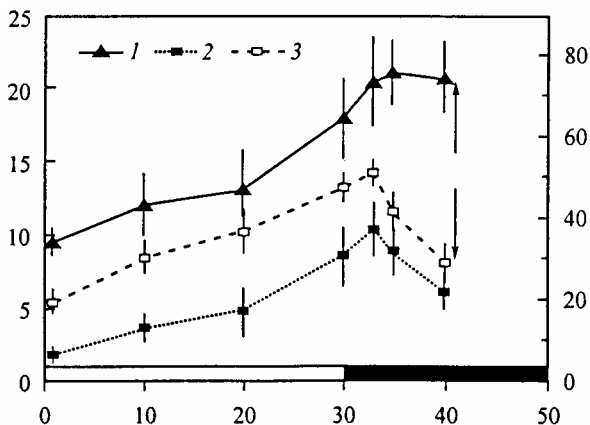


Fig. 6. Dynamics of dry weight, fat weight and relative fat content during induction and termination of reproductive diapause in *Harmonia sedecimnotata* (the second experiment). Ordinate: left, (1) total dry weight (mg), (2) fat weight (mg); right, (3) fat content (%). For other designations, see Fig. 1.

fed on aphids, variations in the percentage of fat during the first 20 days of adult life were insignificant. But when aphids were absent, feeding on the carbohydrate food resulted in the rapid doubling of relative fat content up to 50% and even more. Resumption of feeding on aphids lowered the percentage of fat to the initial level: the difference between the data obtained on the 8th–12th and the 55th days of the experiment (26% and 34%, correspondingly) was insignificant.

In the second experiment, the weight of females fed on only sugar solution increased much slower than that of females fed on aphids (comp. Fig. 3 and Fig. 6). However, when aphids were provided and oviposition was initiated, the total dry weight stabilized at about 20 mg, which is practically equal to the mean weight of ovipositing individuals recorded in the first experi-

ment. Note (Fig. 6) that during the first 30 days of the second experiment, when females were fed on sugar, their weight mostly increased through fat accumulation (during this period, females doubled in the percentage of fat), while onset of feeding on aphids resulted in a sharp decrease in the proportion of fat.

DISCUSSION

Results of our experiments (Fig. 1 and Figs. 3–5, 1) clearly suggest that the onset and termination of oviposition, same as corresponding changes in hermarium size are closely correlated with the dynamics of accumulation and expenditure of fat reserves. It was earlier demonstrated that in some other coccinellids the termination of oviposition caused by the absence of aphids was also accompanied by fat accumulation (Hagen, 1962; Hodek, 1973; Iperti and Hodek, 1974). As noted above, fat accumulation is a usual sign of a diapause, although neurohormonal mechanisms regulating the development of ovaries and the metabolism of lipids may be different (Morita, 1999). Undoubtedly, all the abovementioned processes (onset and termination of oviposition, changes in hermarium size, dynamics of fat reserves) strongly depend on the diet. It is this peculiarity of biology of coccinellids which made it possible to articulate the idea of the “trophic diapause” (Hagen, 1962; Hodek, 1973; Zaslavskiy *et al.*, 1998).

Certainly, in active (not diapausing) insect, both ethological (selectivity of oviposition), and physiological (fecundity) components of reproduction could depend on stimuli connected with a potential host or prey (see reviews: Labeyrie, 1967; Papaj, 2000). In particular, it is known that aphids stimulate oviposition of numerous species of carnivorous coccinellids (Hagen, 1962; Evans, 2003; Evans *et al.*, 2004). Dependence of fecundity on food quality and quantity is also common. For example, *Coleomegilla maculata* Timberlake females terminated oviposition after three days of feeding on the protein-free diet (Smith, 1965). However, this process was not accompanied by an increase in total weight and fat accumulation. Thus, in this case, we are really dealing with a simple interruption of oviposition, but not with a diapause. Similar results were later received in experiments with certain other lady bird species (Dreyer *et al.*, 1997; Richards and Evans, 1998; Evans, 2000, 2003). An overview of experiments with anautogenous Diptera (Barton Browne, 2001) suggests that initiation and termination of oogenesis are regulated by the neuroendocrine sys-

tem, while fecundity is directly dependent on food quality and quantity.

Thus, the results of our experiments with *H. sedecimnotata* and of other studies conducted with carnivorous Coccinellidae suggest the correctness of using the term “diapause” for the termination of oviposition caused by the absence of aphids in the diet of females.

It is well known that the tendency to diapause can be increased by a variety of adverse environmental factors (Danilevskiy, 1961; Tyshchenko, 1983; Tauber *et al.*, 1986; Chernyshev, 1996). V.A. Zaslavskiy repeatedly noted that dependence of insect seasonal development on photoperiod, on temperature, and on other environmental factors (including the “trophic factor”) are based on the same physiological mechanism (Zaslavskiy, 1996; Zaslavskiy *et al.*, 1998). Experiments conducted with *Pyrrhocoris apterus* (L.), a “classical model object” of insect neuroendocrinology, showed that both photoperiod and food effects on oogenesis were equally mediated by juvenile hormone. However, in another representative of the same insect order, *Eurydema rugosa* Motsch., differences between photoperiodically and trophically induced diapauses were revealed (Hodkova, 1999; Musolin and Saulich, 1999).

It has long been known that feeding of phytophagous insects on damaged or stressed plants often caused a decrease in fecundity of ovipositing females, a suppression of oogenesis, and even an early reproductive diapause (Lees, 1956; De Wilde *et al.*, 1969). This effect was also discovered in coccinellids. Females of a phytophagous lady beetle *Epilachna niponica* (Lewis) offered more than half damaged plants, terminated oviposition (egg resorption was recorded), but rapidly resumed it when undamaged plants were provided (Ohgushi and Sawada, 1985; Ohgushi, 1992).

Note that although food influence on the **induction** of reproductive diapause was earlier described for numerous insect species (see above), the influence of diet on the diapause **development** and particularly on the diapause **termination** was much less studied (Hodek, 1999, 2002).

However, results of our and above mentioned similar studies allowed us to make more general conclusions. It is commonly held (e.g., Tauber *et al.*, 1986) that reactions of insects to the environment could be divided into 2 groups, i.e. (1) adaptations to short-term localized changes (easily reversible behavioral and

physiological reactions) and (2) adaptations to long-term seasonal changes, based on neuro-humoral reactions to environmental cues (various forms of diapause). In reality, however, both duration of environmental changes and stability of insect reactions vary gradually.

For example, investigations of the host plant influence on the induction of reproductive diapause in the ragweed leaf beetle, *Zygogramma suturalis* F., showed that during the first days of feeding on damaged ragweed, a direct (vital) reaction to low quality food, namely a decrease in oviposition intensity was observed. At the same time, the movement activity sharply increased (under natural conditions, this means the search for sites with undamaged ragweed). Thus, the decrease in food quality acted also as a cue to behavioral changes. However, after 6–8 days of feeding on damaged ragweed, reproductive diapause was induced in certain females, suggesting that damage to ragweed acted as a cue factor. Note that even short-term interruptions of oviposition were accompanied by thigmotaxis, decreased movement activity, and fat body accumulation (Vinogradova, 1988; Vinogradova and Bogdanova, 1989; Reznik, 1989, 1991; Vinogradova and Pantyukhov, 1995).

Similar results were obtained in studies on Colorado potato beetle, *Leptinotarsa decemlineata* Say (Minder, 1981; Ushatinskaya and Kochetova, 1981). All the components of the “reproductive diapause syndrome” (de Wilde *et al.*, 1969; Hodek, 1999), namely, the oogenesis termination, the metabolism intensity decrease, the accumulation of fat reserves, etc. may develop and disappear during several days. Thus, short-term and reversible termination of oviposition *per se* is not a definite proof of lack of diapause. As rightly noted by V.B. Chernyshev (1996), “there is no sharp distinction between a slight delay in development and a true diapause based on complicated physiological mechanisms.” Recent studies on a fly *Scathophaga stercoraria* (L.) revealed “summer reproductive quiescence,” a drastic inhibition (but not a termination) of reproduction accompanied by the accumulation of fat reserves (Blanckenhorn *et al.*, 2001). A state intermediate between active reproduction and reproductive diapause was also discovered in certain other insects (Pener and Orshan, 1980; Taylor and Schrader, 1984; Numata, 1993).

Egg retention in species of the genus *Trichogramma* Westw. is also “on the verge of diapause.” This relatively well studied phenomenon shows certain proper-

ties peculiar to the reproductive diapause: a qualitative difference between ovipositing and non-ovipositing individuals, an abrupt and irreversible transfer. On the other hand, egg retention differs markedly from diapause in that it is rather short-term and is not accompanied either by significant increase in life duration or by the absence of mature ovarial eggs (Reznik *et al.*, 1998, 2001, 2003).

Thus, results of investigations conducted on carnivorous coccinellids, ragweed leaf beetle, *Trichogramma* species, and some other insects do not fit the traditional subdivision of insect environmental adaptations into "short-term" and "long-term" ones, based, correspondingly, on "neural" and "neurohormonal" mechanisms, once again suggesting that this subdivision is a matter of convention. It should be pointed out that individual adaptations to environmental variations are executed by a gradient or spectrum of simultaneous or sequential reactions. Besides, the boundary between neural and hormonal regulating mechanisms is also conventional (see review: Leibson, 1983).

CONCLUSIONS

(1) It was experimentally demonstrated that onset, termination and resumption of oviposition by *Harmonia sedecimnotata* females, same as corresponding changes of hermarium size are closely correlated with the dynamics of fat reserves accumulation and expenditure.

(2) Even short-term (10 day long) terminations of oviposition caused by the absence of aphids in the presence of carbohydrate food were accompanied by an increase in the absolute and the relative (percentage) fat content. This confirms the correctness of using the term "diapause" for the termination of oviposition caused by the absence of aphids in the diet of Coccinellidae females.

(3) These results and data on the regulation of oviposition in certain other insect species suggest that "short-term" and "long-term" adaptations to the environmental changes are performed by the same mechanism.

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