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Maternal age and endogenous variation in maternal influence on photoperiodic response in the progeny diapause in *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae)

SERGEY YA. REZNIK*, TATYANA S. KATS, TAISIYA YA. UMAROVA and NATALIYA D. VOINOVICH

Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034, Russia; e-mail: reznik@weed.zin.ras.spb.ru

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Abstract. A laboratory study was carried out on photoperiodic control of prepupal diapause in the egg parasitoid *Trichogramma embryophagum* (Hartig). All experiments were conducted with an isofemale parthenogenetic strain. The maternal generation was reared at 20°C and photoperiods of L:D = 3:21, 6:18, 9:15, 12:12, 15:9, 18:6, 21:3 or 24:0. The tendency to diapause in the progeny was estimated by rearing the daughter generation at 15°C in the dark. Experiments revealed a long-day type response based on maternal influence on the progeny prepupal diapause. However, significant endogenous fluctuations in the pattern of the photoperiodic curve were revealed in successive laboratory generations reared under constant conditions. The left threshold day-length was very variable, while the right threshold kept relative constancy. Experiments with individual females sequentially offered new host eggs demonstrated that the probability of the progeny entering diapause depends significantly on maternal age. At 20°C and 18L : 6D, the percentage of diapause was maximal (ca 15%) in the progeny eclosed from the eggs laid during 1^{st} – 2^{nd} days of maternal life. Then the proportion of diapausing progeny decreased to 0–5% at days 9–11 of female life and later slightly increased in 15–17 days old females. Thus, endogenous factors play an important role in maternal influence on progeny diapause, particularly in environments close to threshold temperature and photoperiod.

INTRODUCTION

It is common knowledge that environmental factors, such as photoperiod, temperature, food quality, and population density play a leading role in diapause regulation. However, endogenous components may also be important. Among these is the influence of maternal age on progeny diapause or variation in successive generations of an insect line reared under constant conditions. The environmental control of insect diapause has been the subject of numerous studies, but endogenous factors are much less investigated (Saunders, 1962; Danilevski, 1965; Tauber et al., 1986; Danks, 1987; Zaslavski, 1988).

Our study was conducted with *Trichogramma embryophagum* (Hartig). Numerous species of the genus *Trichogramma* are minute egg parasitoids often used as model insects for various ecological and physiological studies. Moreover, *Trichogramma* species are widely employed for biological control of several pests through inundative releases. Hence, a study of diapause control in this mass reared insect may also be of practical importance. Diapause regulation in *Trichogramma* has already attracted much attention (Boivin, 1994). It was repeatedly demonstrated that prepupal diapause is typical for *Trichogramma* and that temperature is the most important among environmental factors acting on the larvae (Quednau, 1957; Maslennikova, 1959; Bonnemaison, 1972; Voegele, 1976). Later, it was established that, as in certain other egg parasitoids (e.g. Jackson, 1961), this response depends on the conditions of development of the parental generation, and photoperiod is the main factor controlling maternal influence on *Trichogramma* progeny diapause (Zaslavski & Umarova, 1981, 1990; Mai Phu Qui & Zaslavski, 1983; Sorokina & Maslennikova, 1986, 1987). Endogenous diapause-regulating factors have also attracted some attention, particularly changes in the diapause tendency over the course of generations (Zaslavski & Umarova, 1981, 1990).

The present paper deals with:

The influence of maternal age on the tendency of progeny to diapause in *T. embryophagum*.

The variation of maternally operated photoperiodic response in the sequence of generations developing under constant laboratory conditions.

MATERIALS AND METHODS

Insects

A laboratory parthenogenetic strain of *T. embryophagum* originating from Moscow province was cultivated on eggs of the grain moth, *Sitotroga cerealella* Oliv., under constant laboratory conditions (20°C, 18L : 6D) for more than 50 generations. To ensure maximum genetic uniformity, all experiments were conducted with the isofemale sub-strain established from the main strain.

First experiment

For each run of the first experiment, grain moth eggs glued on 32 paper cards (100–200 eggs per card) were placed together in 3-liter glass jars and subjected for 24 h to parasitization by 500–1000 *T. embryophagum* females. Then these cards (the

* Corresponding author.

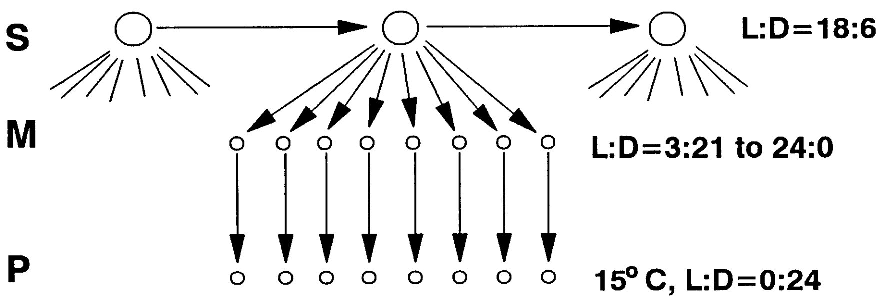

Fig. 1. The design of the first experiment. S – laboratory strain cultivated under constant laboratory conditions (20°C, 18L : 6D); M – maternal generation distributed among eight photoperiods (20°C, 3L : 21D to 24L : 0D); P – progeny generation reared under moderate diapause-inducing conditions (15°C in the dark).

maternal generation) were put in glass tubes and distributed among eight photoperiod regimes at 20°C, L:D= 3:21, 6:18, 9:15, 12:12, 15:9, 18:6, 21:3, and 24:0 (four cards per photoperiod). In 19–20 days, i.e. one day after mass eclosion of the maternal generation, a new card with 500–600 grain moth eggs was placed for 24 h in each of these 32 tubes. Then the newly parasitized host eggs (progeny generation) were placed in moderate diapause-inducing conditions (15°C in the dark). At this temperature, adult eclosion is completed in two months after parasitization. Hence, in two months all parasitized host eggs were dissected, the number of eclosed adults and diapausing prepupae was counted, and the percentage of diapausing individuals was calculated for each card separately. Insects that died during the larval or prepupal stages were excluded from consideration. Each run of the first experiment was conducted with one of the seven successive generations of the *T. embryophagum* strain that was reared under constant diapause-averting conditions: 20°C, 18L : 6D (Fig. 1). Thus, during this experiment 7 generations were tested at 8 photoperiods in 4 replicates (in total, 224 experimental units).

Second experiment

For each run of the second experiment, 1-day old females of the same isofemale line that developed under 20°C and 18L : 6D were placed individually into 40 × 5 mm test tubes, were offered 50–60 host eggs, and were allowed to parasitize during two days. Thereafter, every other day a new supply of fresh host eggs was presented to each surviving female. By the 18^{th} day most females had died and the experiment was terminated. Parasitized host eggs were taken out of the test tubes and, as in the first experiment, placed in moderate diapause-inducing conditions (15°C in the dark). The percentage of diapausing individuals was separately calculated for each portion of eggs successively parasitized by each female. During this experiment, females of 9 generations (25–50 individuals per generation) consecutively parasitized up to 9 portions of host eggs. The females which refused to oviposit or laid less than 10 eggs (30 of 250 wasps) were excluded from the statistical treatment. Thus, the final sample size was 1186 experimental units (portions of host eggs).

It should be noted that under these conditions (maternal photoperiod 18L : 6D) the tendency to diapause is weak (see Fig. 2). However, in the study of age-dependence this photoperiod (under which the main *Trichogramma* strain was reared during numerous generations before the experiment) was used with the aim of precluding interference with the processes caused by photoperiod change. It is known (Mousseau & Dingle, 1991; Vinogradova, 1991) that in insect species manifesting maternal influence on progeny diapause, a change of environmental conditions often causes long-term impact that can be followed for many generations.

Statistical treatment

To stabilize variance, the percentages of diapausing individuals were arcsine-transformed (Lloyd & Ledermann, 1984) before mean and SEM calculation, ANOVA, and Tukey HSD test. All tests were performed using SYSTAT (Wilkinson, 1990).

RESULTS

First experiment

The percentage of diapausing progeny depends on both the maternal photoperiod ($F = 171.3$, $p < 0.001$) and on the generation under study ($F = 18.9$, $p < 0.001$). It is evident from Fig. 2 that *T. embryophagum* manifested a long-day type response. The maximum rate of progeny diapause (20– 45%) was recorded at a day length of 12 h. The threshold photophase, defined as the day length inducing half of maximum diapause percentage in a given generation, ranged from 6 to 10 h (left threshold) and from 16 to 17 h (right threshold). Notice the sharp difference in the range of variation of the threshold photophase between the left and the right threshold. Note also the difference in the coefficient of variation of progeny diapause between the left and the right threshold zone (Fig. 2).

Second experiment

Practically all females of the *T. embryophagum* strain under study started parasitization during the first two days of the experiment. In this period, 14.7 ± 0.4 eggs/female/48 h were laid (hereafter, mean±SEM is given). During the 3^{rd}–18^{th} days of a female's life, the intensity of oviposition slightly decreased from 7–8 to 5–6 eggs/female/48 h. Total lifetime fecundity was 46.0 ± 1.5 eggs/female.

As expected based on the results of the first experiment, the tendency to diapause was low. The percentage of diapausing progeny estimated for the total sum of data varied from 15% in the progeny of young females to 0–5% with 9–11 days old females. The ANOVA test of arcsine transformed data (n = 1186) demonstrated that the percentage of diapausing progeny depends both on maternal age ($F = 13.0$, $p < 0.001$) and on the generation under study ($F = 16.3$, $p < 0.001$). For the further analysis of age-dependence, the arcsine transformed data were trans-

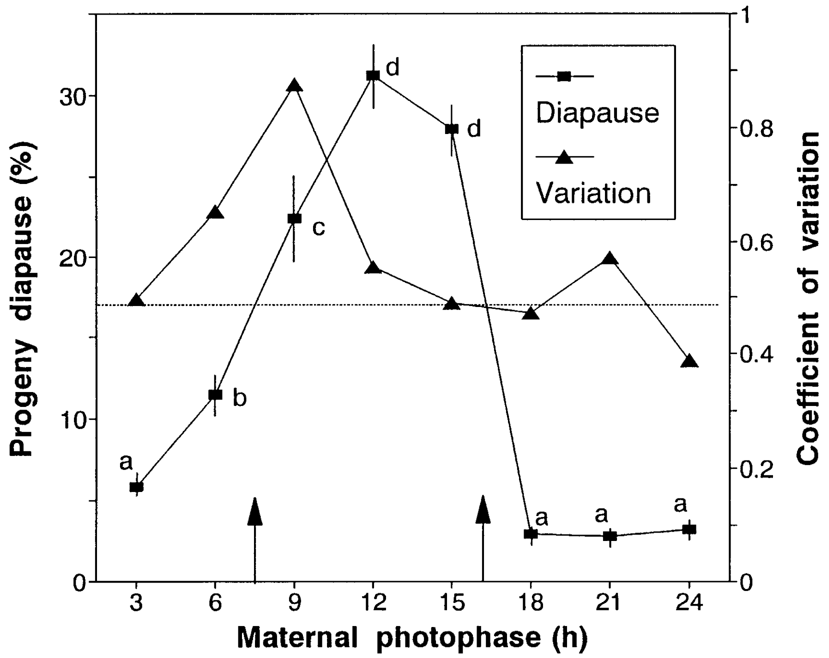

Fig. 2. The effect of the maternal photoperiod on the percentage of progeny diapause (given are the means, vertical bars indicate ± SEM) and its coefficient of variation in the sequence of laboratory generations. Means accompanied with different letters are significantly different at the $p < 0.05$ level (Tukey HSD test). Dashed line indicates a half of maximum diapause percentage. Arrows indicate threshold photophases.

formed once again. To exclude the "generation factor", each value was replaced by its relative deviation from the mean in the given generation:

Y = (X i – X mean) / X mean

where X i – arcsine transformed percentage of diapausing progeny produced by a given female at a given age; X mean – mean arcsine transformed percentage of total diapausing progeny produced by all females of a given generation.

It is clear from the twice transformed data (Fig. 3) that the relative tendency to prepupal diapause was maximal in the progeny eclosed from the eggs laid during the first two days of maternal life. Then the proportion of diapausing progeny decreased, but seems to increase again in 15–17 day old females.

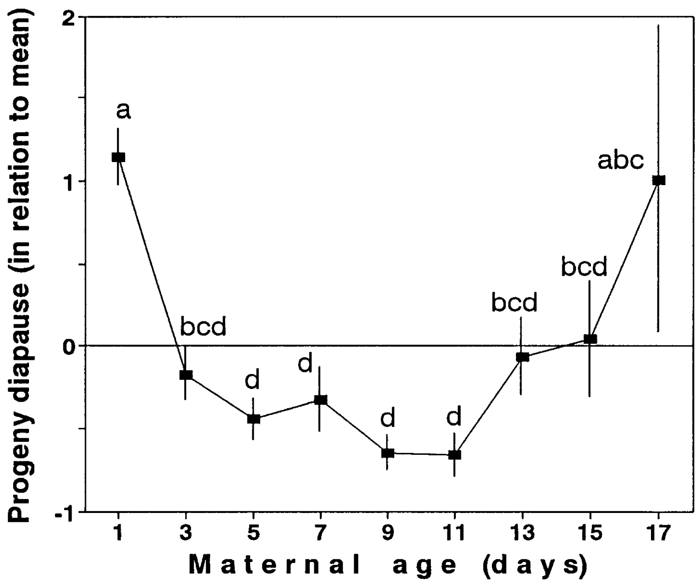

Fig. 3. The effect of maternal age on the proportion of progeny in diapause (in relation to the mean overall for the given generation). Given are the means; vertical bars indicate ± SE. Means accompanied by different letters are significantly different at the $p < 0.05$ level (Tukey HSD test).

DISCUSSION

The results of the first experiment agree well with the earlier published data on *T. embryophagum*. It was demonstrated (Sorokina & Maslennikova, 1986, 1987) that a maternal photoperiod of 10L : 14D induced diapause in 10% of progeny developing at 15°C, while with 16L : 8D, diapausing individuals were practically absent (other photoperiods were not tested in the quoted work). The only known study conducted with *Trichogramma* along the whole photoperiodic scale, revealed a similar long-day type maternally operated photoperiodic reaction in *T. pintoi* Voegele (Zaslavski & Umarova, 1990).

Endogenous increases and decreases in the tendency to diapause were repeatedly observed in laboratory lines of different insect species (Geyspits et al., 1978; Vinogradova & Bogdanova, 1980; Zaslavski & Umarova, 1981; Vinogradova & Reznik, 2000). The stability of the right threshold of the photoperiodic curve (compared to the left threshold) was earlier recorded in a comparison between the photoperiodic responses manifested at different temperatures and, thus, was often considered as a specific feature of the temperature dependence of the photoperiodic reaction (Tyshchenko, 1977; Saulich, 1999). However, it seems to be a more general phenomenon. In our study conducted at constant temperature, the left threshold day-length was also very variable, while the right threshold day-length was relatively constant. A possible reason is that the right threshold is subject to stabilizing selection under natural conditions, while the left threshold zone is a selectively neutral character revealed only in laboratory experiments (Danilevski, 1965; Zaslavski, 1988).

In most of the previously investigated insects, the percentage of progeny diapause increased with female age (Saunders, 1962; Ring, 1967). Less commonly, this age-dependence was negative (Verdier, 1970) or chaotic (Saunders, 1965; Mousseau & Dingle, 1991; Vinogradova, 1991). In certain studies, the sign of age-dependence was controlled by photoperiod (Saunders, 1987; Vinogradova & Reznik, 1999). Complicated dependence of the tendency to diapause (namely, of the proportion of oviparous females which lay diapausing eggs) on the maternal age, similar to our results, was recently described in the pea aphid *Acyrthosiphon pisum* Harris (Erlykova, 1997, 1999).

Thus, the role of an endogenous component in the maternal control of progeny diapause in insects is convincingly confirmed by the new experimental data. In particular, the dependence of progeny diapause on maternal age and the difference in stability between the left and the right threshold of the photoperiodic response have been demonstrated in *Trichogramma* for the first time. We conclude that a possible role for an endogenous component in the regulation of insect seasonal cycles (especially manifested in the neighborhood of threshold temperature and photoperiodic conditions) should be taken into account in further studies on insect ecology and physiology.

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REFERENCES

BOIVIN G. 1994: Overwintering strategies of egg parasitoids. In: Wajnberg E. & Hassan S.A. (eds): *Biological Control with Egg Parasitoids*. CAB International, Wallingford, UK, pp. 219–244.

BONNEMAISON L. 1972: Diapause et superparasitisme chez Trichogramma evanescens Westw. (Hymenoptera: Trichogrammatidae). *Bull. Soc. Entomol. France* **77**: 122–132.

DANILEVSKI A.S. 1965: *Photoperiodism and Seasonal Development of Insects*. Oliver & Boyd, London, 283 pp.

DANKS H.V. 1987: Insect dormancy: an ecological perspective. *Biological Survey of Canada Monograph* **1**: 1–439.

ERLYKOVA N.N. 1997: Peculiarities of the photoperiodic reaction of the unisexual clone of the pea aphid Acyrthosiphon pisum Harris (Homoptera: Aphididae). *Entomol. Obozr.* **76**: 497–507. [English translation: *Entomol. Review* **76**: 301–308]

ERLYKOVA N.N. 1999: Effect of photoperiod and maternal age on the progeny pattern of the pea aphid *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) from the Volga area. *Entomol. Obozr.* **78**: 275–286. [English translation: *Entomol. Review* **79**: 1098–1106]

GEYSPITS K.F., GLINYANAYA E.I., DUBININA T.S., KVITKO N.V., PIDZHAKOVA T.V., RAZUMOVA A.P. & SAPOZHNIKOVA F.D. 1978: The annual endogenous rhythm of changes in the photoperiodic reaction of arthropods and its relation to exogenous factors. *Entomol. Obozr.* **57**: 731–745. [English translation: *Entomol. Review* **57**: 495–505]

JACKSON D.J. 1961: Diapause in aquatic mymarid. *Nature* **192**: 823–824.

LLOYD E. & LEDERMANN W. (eds) 1984: *Handbook of Applicable Mathematics*. Vol. VI: *Statistics*. John Wiley & Sons Ltd., N.Y., 510 pp.

MAI PHU QUI & ZASLAVSKI V.A. 1983: Photoperiodic and temperature reactions in Trichogramma euproctidis (Hymenoptera: Trichogrammatidae). *Zool. Zhurn.* **62**: 1676–1680. [in Russian]

MASLENNIKOVA V.A. 1959: On the wintering and diapause in Trichogramma evanescens Westw. *Vestnik Leningradsk. Universiteta* **3**: 91–96. [in Russian]

MOUSSEAU T.A. & DINGLE H. 1991: Maternal effects in insect life histories. *Annu. Rev. Entomol.* **36**: 511–534.

QUEDNAU W. 1957: Über den Einfluss von Temperatur und Luftfeuchtigkeit auf den Eiparasiten Trichogramma cacoeciae Marchal. *Mitt. Biol. Bundesanst. Land- und Forstwirtsch.* **90**: 1–63.

RING R.A. 1967: Maternal induction of diapause in the larva of Lucilia caesar L. (Diptera: Calliphoridae). *J. Exp. Biol.* **46**: 123–136.

SAULICH A.K. 1999: *Insect Seasonal Development and Dispersal Potentialities*. St. Petersburg State University Press, St. Petersburg, 246 pp. [in Russian]

SAUNDERS D.S. 1962: The effect of the age of female Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) upon the incidence of larval diapause. *J. Insect Physiol.* **8**: 309–318.

SAUNDERS D.S. 1965: Larval diapause of maternal origin: Induction of diapause in Nasonia vitripennis Walk. (Hymenoptera: Pteromalidae). *J. Exp. Biol.* **42**: 495–508.

SAUNDERS D.S. 1987: Maternal influence on the incidence and duration of larval diapause in Calliphora vicina. *Physiol. Entomol.* **12**: 331–338.

SOROKINA A.P. & MASLENNIKOVA V.A. 1986: The peculiarities of phototermic reactions in certain species of the genus Trichogramma (Hymenoptera: Trichogrammatidae). *Vestnik Leningradsk. Gos. Universiteta, Series 3*, **1**: 9–14. [in Russian]

SOROKINA A.P. & MASLENNIKOVA V.A. 1987: Temperature optimum for diapause determination in species of the genus Trichogramma Westw. (Hymenoptera: Trichogrammatidae). *Entomol. Obozr.* **66**: 689–699. [in Russian]

TAUBER M.J., TAUBER C.A. & MASAKI S. 1986: *Seasonal Adaptations of Insects*. Oxford University Press, New York, Oxford, 411 pp.

TYSHCHENKO V.P. 1977: Physiology of insect photoperiodism. *Trud. Vses. Entomol. Obshch.* **59**: 1-155. [in Russian]

VERDIER M. 1970: Diapause embryonnaire de Locusta des latitudes basses: influence de l'age des parents et de la photoperiode. *C.R. Acad. Sci. (Paris), Ser. D*, **270**: 148–151.

VINOGRADOVA E.B. 1991: *Diapause in Flies and its Control.* Nauka, St. Petersburg, 253 pp. [in Russian]

VINOGRADOVA E.B. & BOGDANOVA T.P. 1980: The endogenous cyclic changes of the tendency to diapause in the strains of flesh flies and blow flies, developing continuously at the constant conditions. *Entomol. Obozr.* **59**: 26–38. [in Russian]

VINOGRADOVA E.B. & REZNIK S.YA. 1999: Endogenous changes of the tendency to diapause in the blowfly, Calliphora vicina (Diptera: Calliphoridae). *Proc. Zool. Inst. Russian Acad. Sci.* **281**: 151–155.

VINOGRADOVA E.B. & REZNIK S.YA. 2000: Endogenous changes of the tendency to larval diapause in laboratory generation sequences of the blowfly, Calliphora vicina R.-D. (Diptera: Calliphoridae). *Intern. J. Dipterol. Res.* **11**: 3–8.

VOEGELE J. 1976: La diapause et l'heterogenite du developpement chez les *Aelia* (Heteroptera: Pentatomidae) et les Trichogrammes (Hymenoptera: Trichogrammatidae). *Ann. Zool. Ecol. Anim.* **8**: 367–371.

WILKINSON L. 1990: SYSTAT: The System for Statistics. Systat Inc., Evanston, IL, USA, 631 pp.

ZASLAVSKI V.A. 1988: *Insect Development: Photoperiodic and Temperature Control.* Springer-Verlag, N.Y., Berlin, 187 pp.

ZASLAVSKI V.A. & UMAROVA T.YA. 1981: Photoperiodic and temperature control of diapause in Trichogramma evanescens Westw. (Hymenoptera, Trichogrammatidae). *Entomol. Obozr.* **60**: 721–731. [English translation: *Entomol. Review* **60**: 1–12]

ZASLAVSKI V.A. & UMAROVA T.YA. 1990: Environmental and endogenous control of diapause in Trichogramma species. *Entomophaga* **35**: 23–29.

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