On the Nature of Individual Variation in Diapause Induction among the Progeny of *Trichogramma telengai* Sor. (Hymenoptera, Trichogrammatidae)

N. D. Voinovich and S. Ya. Reznika,*

*a* Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034 Russia

*e-mail: reznik1952@mail.ru*

Received January 20, 2022; revised January 26, 2022; accepted January 26, 2022

**Abstract**—In this communication, we analyze the variation in diapause incidence among isofemale substrains of the main laboratory strain of *Trichogramma telengai* Sor. (Hymenoptera, Trichogrammatidae) in order to clarify the nature of the previously discovered individual (intrastrain) variation in this trait. During the experiment, the maternal generation developed at a temperature of 20°C under a day length of either 12 or 18 h, and diapause in the progeny was induced by development at 14°C in darkness. In total, the experiment comprised eight replicates in which female diapause incidence was measured in 428 substrains in two consecutive generations. There was statistically significant individual (first-generation) and interfamilial (second-generation) variation in almost all of the replicates. However, the correlation between the diapause incidence values among the progeny of two consecutive generations of the same substrains was weak and largely nonsignificant. These results suggest that intrastrain variation in the percentage of diapausing progeny of *T. telengai* females is not primarily produced by genetic (hereditary) factors but is due to certain environmental (nonhereditary) effects.

**Keywords:** diapause, variation, inheritance, biocontrol, *Trichogramma telengai*, Trichogrammatidae

**DOI:** 10.1134/S0013873822010031

Individual variation, which is observed within natural populations or within laboratory-reared and industrial strains, affects, to a varying degree, all the morphological, physiological and behavioral traits of insects. Non-hereditary (environmentally induced) variation is the key mechanism of adaptation to relatively short-term changes and spatial heterogeneity of the environment. Hereditary individual variation is the necessary prerequisite to both natural evolution of insects and artificial selection of new lines and varieties of beneficial species. However, uncontrolled intrastrain variation during laboratory and industrial rearing makes it more difficult to carry out research and standardize methods, which not infrequently results in decreased quality of biocontrol agents. Thus, the diverse forms of individual variation warrant further study as being of interest to both basic and applied entomology.

In particular, most insects studied for facultative diapause induction exhibit a more or less substantial intra-specific variation in their responses to diapause-inducing photoperiods and temperatures. Diapause is among the most important ecophysiological adaptations and synchronizes insect seasonal cycles of activity with the dynamics of environmental factors. Facultative diapause usually commences prior to the onset of adverse conditions and is induced by seasonal cues such as photoperiod (day length), temperature, diet quality, etc. Intraspecific variation, both within (individual) and between populations, which underpins the plasticity of diapause responses, provides the basis for risk-spreading strategies and microevolution of seasonal adaptations in insects (Danilevskii, 1961; Zaslavski, 1984; Tauber et al., 1986; Saulich, 1999; Denlinger, 2002; Saunders et al., 2002; Saulich and Volkovich, 2004; Danks, 2007; Tougeron, 2019; Saunders, 2020; Snell-Rood and Ehlman, 2021).

Species of the genus *Trichogramma* Westw. (Hymenoptera, Trichogrammatidae) are minute parasitoids of
The observed variation is due to nonhereditary processes. Whether this variation is genetic or due to nonhereditary factors is an open question. The present study aims at finding out the nature of this variation, however, remained an open question. The present study aims at finding out whether this variation is genetic or due to nonhereditary factors.

The experimental design was quite simple: we studied individual variation in diapause incidence among the progeny over two consecutive generations of isofemale substrains isolated from the main laboratory strain. The presence of a positive correlation between the two generations of the same substrain would indicate that the differences between maternal females are mostly genetic, whereas the absence thereof would suggest that the observed variation is due to nonhereditary processes.

MATERIALS AND METHODS

We used the same parthenogenetic laboratory strain of *Trichogramma telengai* as in the previous work (Reznik and Voinovich, 2019). Prior to the experiments, wasps had been reared on eggs of the grain moth *Sitotroga cerealella* (Oliv.) (Lepidoptera, Gelechiidae) under constant laboratory conditions (20°C, 18 h of light) for many years.

Each experimental replicate started with grain moth eggs being pasted with polyvinyl acetate glue onto cardboard cards and presented for 24 h to laboratory strain wasps for oviposition. Immediately following this, each card with parasitized host eggs was divided into two halves, which were individually incubated in large tubes (10 cm in length, about 3 cm in diameter) under a photoperiod of either 12:12 or 18:6 (light:darkness, h). The temperature in both photoperiodic regimens was the same at 20°C. On the day of mass emergence of adult wasps of this (first maternal) generation, females were individually allocated to small (5 cm in length, about 0.8 cm in diameter) numbered tubes with 50% honey solution applied to the walls as a source of carbohydrates. The progeny of each female from this first generation was considered as representing a substrain. Immediately after allocation to the individual tubes, the females of the first maternal generation were presented with numbered cards that contained 30–40 grain moth eggs. After 2 h, the females were returned to the photoperiodic regimens they had developed in and the cards with parasitized eggs (the first daughterly generation) were transferred to moderately diapause-inducing conditions (darkness, 14°C). After 2 days, females of the first maternal generation were presented for 24 h with numbered cards that contained the second portion of grain moth eggs (about 50 eggs per card). The cards with parasitized eggs (the second maternal generation) were transferred to individual tubes of the same size and incubated in the same regimen as the previous generation (either 12-h or 18-h light at 20°C).

On the day of mass emergence of adult wasps of the second maternal generation, females were kept in the same tubes they had developed in (depending on the maternal fecundity, the number of wasps per tube usually varied from 5 to 15 ind.) and presented for 2 h with numbered cards that contained 400–600 grain moth eggs. The cards with parasitized eggs (the second daughterly generation) were transferred to moderately diapause-inducing conditions (darkness, 14°C).

Eggs and serve as natural enemies to many lepidopteran pests of agriculture and forestry. These wasps are widely used as biological control agents in plant protection (Smith, 1996; Sorokina, 2011). Facultative prepupal winter diapause in *Trichogramma* is primarily controlled by temperature, but at near-threshold temperatures, the percentage of diapausing progeny strongly depends on the photoperiodic conditions experienced by the maternal generation (Zaslawski and Umarova, 1981; Mai Phu Qui and Zaslawski, 1983; Zaslawski and Umarova, 1990; Boivin, 1994; Reznik, 2011). Different forms of interpopulational (interstrain) variation in photoperiodically and thermally induced diapause responses were discovered in many species of *Trichogramma* (Sorokina, 1987; Sorokina and Maslennikova, 1987; Sorokina, 2010). However, to the best of our knowledge, no dedicated analysis of intrapopulational (intrasubstrain) variation has been carried out before our previous work (Reznik and Voinovich, 2019). The latter study showed that the parthenogenetic laboratory strain of *Trichogramma telengai* Sor. harbored substantial variation between females in the percentage of diapause in their progeny. The variation in diapause incidence was statistically significant in most replicates of the experiment, including the cases where the total fraction of diapausing individuals approached 90%. The distribution of females by the percentage of diapausing progeny was always unimodal, indicative of a quantitative (not qualitative), or continuous, type of individual variation. There was a significant positive correlation between diapause incidence values in the progeny of the same wasp female that consecutively parasitized different egg batches of the host species, and thus individual properties of the females explained a large portion of the variation observed. The nature of this variation, however, remained an open question. The present study aims at finding out whether this variation is genetic or due to nonhereditary factors.

The experimental design was quite simple: we studied individual variation in diapause incidence among the progeny over two consecutive generations of isofemale substrains isolated from the main laboratory strain. The presence of a positive correlation between the two generations of the same substrain would indicate that the differences between maternal females are mostly genetic, whereas the absence thereof would suggest that the observed variation is due to nonhereditary processes.
In order to accelerate development and adult emergence in nondiapausing individuals, 30 days after parasitization, cards with wasps of the first and second daughterly generations were transferred to environmental chambers set to 20°C and an 18-h day length. After 15–20 d (when the majority of actively developing individuals would have emerged), all the parasitized eggs were dissected to count the number of actively developing (adults and pupae) and diapausing (prepupae) progeny. The number of adults emerged was estimated based on the number of empty eggshells with emergence holes. Larvae that died at different developmental stages were dismissed. The first and second daughterly generation cards that had less than 5 survivors (either diapausing or active) were discarded. The final data set only contained the substrains for which diapause incidence was known in both consecutive generations. Thus, in 8 experimental replicates, we were able to collect data on the progeny of two consecutive generations of 428 substrains of *T. telengai*. It should be noted that in the first maternal generation, each substrain was represented by a single female and in the second maternal generation, by several females. The counts of actively developing and diapausing wasps in the first and second daughterly generations yielded a total of 24,069 ind. The separate sample sizes for the different replicates and photoperiodic regimens are summarized in Fig. 1 and Fig. 2.

The experimental replicates were never synchronous and, as a rule, contained individuals from different generations of the laboratory culture. Previous studies showed that the percentage of diapausing individuals varied noticeably from generation to generation in different strains and species of *Trichogramma* wasps, even under constant conditions (Zaslavski and Umarova, 1981; Voinovich et al., 2013; Reznik et al., 2015). Therefore, data from different replicates were analyzed separately and independently of each other. Significance of individual variation was tested with a chi-square test. Correlations were analyzed with ordinary linear regression. All analyses were performed in SYSTAT 10.2.

**RESULTS AND DISCUSSION**

In the vast majority of experimental replicates under short-day conditions (Fig. 1) and in all of the long-day replicates (Fig. 2), there was statistically significant individual variation in diapause incidence between the progeny of *T. telengai* females in the first and second maternal generations. Furthermore, as can be seen in the figures, long-day conditions resulted not only in higher significance values but also in a broader variation range. These findings confirm the results of our previous study (Reznik and Voinovich, 2019) where the range of individual variation also depended on the photoperiod and temperature experienced by the maternal and daughterly wasp generations. In nature, greater individual variance in the tendency to enter diapause under long-day conditions may contribute to a risk-spreading strategy whereby, under short-day photoperiods of autumn, before the onset of winter, diapause is induced in all or at least in the vast majority of wasps, but some individuals tend to enter diapause under long-day conditions, i.e., in summer, when average climatic variables still permit successful completion of one or more additional generations. Thus, the population has a greater chance of survival in case of early cold snaps or other extreme deviations from the climatic norm. Such individual variation of seasonal cycles was discovered, e.g., in the ragweed leaf beetle *Zygogramma suturalis* (F.) (Coleoptera, Chrysomelidae) (Vinogradova, 1988) and in many other insect species (Hopper, 1999; Joschinski and Bonte, 2021).

The results of our experiment suggest that the variation observed is mostly nonhereditary as the correlation between the diapause incidence values in two consecutive generations of the same substrains was nonsignificant in the vast majority of cases (Fig. 1 and Fig. 2). However, in two out of eight long-day replicates, where variation was more pronounced, the correlation was positive and significant (Fig. 2, replicate A) or even highly significant (Fig. 2, replicate C). Apparently, hereditary factors do contribute to the individual variation in the percentage of diapausing progeny between wasp females, but these hereditary differences can only be detected in the absence of substantially stronger confounding extrinsic factors, which were uncontrolled in our study. The nature of these factors remains obscure. The main abiotic factors that may have influenced the induction of diapause were controlled precisely: day length was set accurate to a few minutes, and temperature deviated from the set value by no more than 0.2–0.3°C. Furthermore, these deviations in conditions were the same for all of the individuals in a replicate and may have shifted the mean values or create differences be-
Fig. 1. Individual variation in diapause incidence among the progeny of short-day (12 h) reared Trichogramma telengai Sor. females. F1 and F2 columns: abscissa – percentage of diapausing progeny, mid-class values are shown; ordinate – distribution of females of different classes according to diapause incidence in their progeny. F1 – first generation of substrains (n₁ – number of maternal females, n₂ – number of daughters studied, p – significance of differences between the females according to chi-squared test). F2 – second generation of substrains (n₁ – number of substrains, n₂ – number of daughters studied, p – significance of differences between the substrains according to chi-squared test). F1–F2 column: abscissa – percentage of diapausing females of the first generation; ordinate – percentage of diapausing females of the second generation of the same substrains (r – correlation coefficient, n – number of substrains, p – significance of correlation), each symbol corresponds to one substrain, the line illustrates the linear regression equation. Rows A–H – experimental replicates.
Fig. 1. (Contd.)
Fig. 2. Individual variation in diapause incidence among the progeny of long-day (18 h) reared *Trichogramma telengai* Sor. females. Designations as in Fig. 1.
Fig. 2. (Contd.)
tween replicates but could not be responsible for differences between individuals and substrains, which were compared only within replicates.

As mentioned above, many insect species have been shown to exhibit interpopulational (in nature) and intrastain (in the laboratory) variation in the tendency to enter diapause (Danilevskii, 1961; Zaslavski, 1984; Tauber et al., 1986; Saulich, 1999; Denlinger, 2002; Saunders et al., 2002; Saulich and Volkovich, 2004; Danks, 2007; Tougeron, 2019; Saunders, 2020). In nature, differences between populations usually correlate with local dynamics of the main environmental factors (temperature, availability of food, etc.) and often show clinal patterns. Individual (intrapopulational and intrastain) variation of diapause responses has been more rarely studied but, without doubt, is similarly widespread, as accumulation of interpopulational differences would not be possible in the absence of individual variation. It should be noted that the studies cited above mostly deal with natural populations or laboratory strains of bisexual insect species, while our work addresses a parthenogenetic strain where one would expect to find lower individual variation. Even in this respect, however, T. telengai is by no means an exception. For example, the pea aphid Acyrthosiphon pisum (Harris) (Hemiptera, Aphididae) was shown to have not only interclonal but also intrACLonal variation in photoperiodic reactions controlling its seasonal cycle (Erylykova, 2003).

In nature, individual variation plays a beneficial role in insects as it increases the plasticity, evolutionary potential, and environmental robustness of their populations. By contrast, intrastain variation of biocontrol agents not only provides material for selection but often appears to be a significant negative factor that hampers standardization of the reared stock, which must be taken into account in the development of industrial and laboratory rearing methods (Hoy, 1986; Hopper, 1999; Wajnberg, 2004; Routray et al., 2016; Bielza et al., 2020; Leung et al., 2020; Joschinski and Bonte, 2021).

ACKNOWLEDGMENTS

The authors are deeply grateful to T. Ya. Umarova (Zoological Institute, RAS) for her help with experimental work.

FUNDING

The work was carried out as part of the state contract no. 1021051302540-6 “Systematics, morphology, ecophysiology, and evolution of insects.”

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All the applicable international, national, and institutional guidelines for the care and use of animals were followed. All the procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

OPEN ACCESS

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

REFERENCES

Bielza, P., Balanza, V., Cifuentes, D., and Mendoza, J.E., Challenges facing arthropod biological control: identifying traits for genetic improvement of predators in protected crops, Pest Management Science, 2020, vol. 76, no. 11, p. 3517.

Boivin, G., Overwintering strategies of egg parasitoids, in Biological Control with Egg Parasitoids, Wajnberg, E. and Hassan, S.A., Eds., Wallingford: CAB Int., 1994.

Danilevskii, A.S., Fotoperiodizm i Sezonnoe Razvitie Nasekomых (Photoperiodism and Seasonal Development of Insects), Leningrad: LGU, 1961.

Danks, H.V., The elements of seasonal adaptations in insects, Can. Entomol., vol. 139, no. 1, p. 1.

Denlinger, D.L., Regulation of diapause, Annu. Rev. Entomol., 2002, vol. 47, p. 93.

ENTOMOLOGICAL REVIEW Vol. 102 No. 1 2022
Erlykova, N.N., Inter- and intraclonal variability in the photoperiodic response and fecundity in the pea aphid Acyrthosiphon pisum (Hemiptera: Aphididae), Eur. J. Entomol., vol. 100, no. 1, p. 31. https://doi.org/10.14411/eje.2003.006

Hopper, K.R., Risk-spreading and bet-hedging in insect population biology, Annu. Rev. Entomol., 1999, vol. 44, p. 535. https://doi.org/10.1146/annurev.ento.44.1.535

Hoy, M.A., Use of genetic improvement in biological control, Agric. Ecosyst. Environ., 1986, vol. 15, no. 2–3, p. 109. https://doi.org/10.1016/0167-8809(86)90084-8

Joschinski, J. and Bonte, D., Diapause and bet-hedging strategies in insects: a meta-analysis of reaction norm shapes, Oikos, 2021, vol. 130, no. 8, p. 1240. https://doi.org/10.1111/oik.08116

Leung, K., Ras, E., Ferguson, K.B., et al., Next-generation biological control: the need for integrating genetics and genomics, Biol. Rev., 2020, vol. 95, no. 6, p. 1838. https://doi.org/10.1111/brv.12641

Mai Phu Qui and Zaslavski, V.A., Photoperiodic and temperature responses of Trichogramma euproctidis (Hymenoptera, Trichogrammatidae), Zool. Zh., 1983, vol. 62, no. 11, p. 1676.

Reznik, S.Ya., Ecological and evolutionary aspects of photothermal regulation of diapause in Trichogramma, Zh. Evol. Biokhim. Fiziol., 2011, vol. 47, no. 6, p. 434.

Reznik, S.Ya. and Voinovich, N.D., Individual variation in progeny diapause induction in Trichogramma telengai Sor. females (Hymenoptera, Trichogrammatidae), Entomol. Obozr., 2019, vol. 98, no. 1, p. 5. https://doi.org/10.1134/S0367144519010015

Reznik, S.Ya., Vaghina, N.P., and Voinovich, N.D., Variations in the tendency to diapause among successive generations of laboratory strains of Trichogramma species (Hymenoptera, Trichogrammatidae): endogenous or exogenous? Zool. Zh., 2015, vol. 94, no. 4., p. 446.

Routray, S., Dey, D., Baral, S., Das, A.P., and Mahantheswar, B., Genetic improvement of natural enemies: a review, Agric. Rev., 2016, vol. 37, no. 4, p. 325. http://dx.doi.org/10.18805/ag.v37i4.6463

Saulich, A. Kh., Sezonnoe razvitie nasekomykh i vozmozhnosti ikh rasseleiniya (Seasonal Development of Insects and Possibilities for their Dispersal), St. Petersburg, SPbGU, 1999.

Saulich, A. Kh. and Volkovich, T.A., Ekologiya fotoperiododznia nasekomykh (Ecology of Insect Photoperiodism), St. Petersburg, SPbGU, 2004.

Saunders, D.S., Insect photoperiodism: seasonal development on a revolving planet, Eur. J. Entomol., 2020, vol. 117, no. 1, p. 328. https://doi.org/10.14411/eje.2020.038

Saunders, D.S., Steel, C.G.H., Vafopoulou, X., and Lewis, R.D., Insect Clocks, Amsterdam: Elsevier, 2002.

Smith, S.M., Biological control with Trichogramma: advances, successes, and potential of their use, Annu. Rev. Entomol., 1996, vol. 41, p. 375. https://doi.org/10.1146/annurev.en.41.010196.002111

Snell-Rood, E.C. and Ehlmam, S.M., Ecology and evolution of plasticity, in Phenotypic Plasticity and Evolution, Pfennig, D.W., Ed., Boca Raton: CRC Press, 2021.

Sorokina, A.P., Biological and morphological justification of species status of Trichogramma telengai sp. n. (Hymenoptera, Trichogrammatidae), Entomol. Obozr., 1987, vol. 66, no. 1, p. 32.

Sorokina, A.P., Photothermal reactions that control diapause in three species of Trichogramma (Hymenoptera, Trichogrammatidae) from Leningrad Province, Vest. Zashch. Rast., 2010, vol. 3, p. 51.

Sorokina, A.P., The use of Trichogramma: past and present, Zashch. Karantin Rast., 2011, vol. 10, p. 9.

Sorokina, A.P. and Maslennikova, K.A., The temperature optimum for the onset of diapause in species of Trichogramma Westw. (Hymenoptera, Trichogrammatidae), Entomol. Obozr., 1987, vol. 66, no. 4, p. 689.

Tauber, M.J., Tauber, C.A., and Masaki, S., Seasonal Adaptations of Insects, New York: Oxford Univ., 1986.

Tougeron, K., Diapause research in insects: historical review and recent work perspectives, Entomol. Exp. Appl., 2019, vol. 167, no. 1, p. 27. https://doi.org/10.1111/eea.12753

Vinogradova, E.B., Peculiarities of reproduction and forms of imaginal diapause in the ragweed leaf beetle Zygogramma celtici (Coleoptera, Chrysomelidae), Entomol. Obozr., 1988, vol. 67, no. 3, p. 468.

Voinovich, N.D., Reznik, S.Ya., and Vaghina, N.P., Variation in the “spontaneous” dynamics in the tendency to diapause in consecutive generations of Trichogramma telengai Sor., Entomol. Obozr., 2013, vol. 92, no. 3, p. 465.

Wajnberg, E., Measuring genetic variation in natural enemies used for biological control: why and how? in Genetics, Evolution and Biological Control, Ehler, L.E., Sforza, R., and Mateille, T., Eds., Wallingford: CAB Int., 2004. http://dx.doi.org/10.1079/9780851997353.0019

Zaslavski, V.A., Fotoperiodicheskii i temperaturnyi kontrol’ razviitii nasekomykh (Photoperiodic and Temperature Control of Insect Development), Leningrad: Nauka, 1984.

Zaslavski, V.A. and Umarova, T. Ya., Control of diapause in Trichogramma evanescens Westw. (Hymenoptera, Trichogrammatidae) by temperature and photoperiod, Entomol. Obozr., 1981, vol. 60, no. 4, p. 721.

Zaslavski, V.A. and Umarova, T.Ya., Environmental and endogenous control of diapause in Trichogramma species, Entomophaga, 1990, vol. 35, no. 1, p. 23. https://doi.org/10.1007/BF02374297