

Influence of a Single (Stepwise) Change in Photoperiod and Female Age on Larval Diapause in the Blowfly *Calliphora vicina* R.-D. (Diptera, Calliphoridae)

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Abstract—In the blowfly *Calliphora vicina*, the induction of the larval diapause is under double control, i.e., maternally operating photoperiod determines the progeny inclination to the diapause, which is manifested depending on temperature. The experiments with a single stepwise change of the photoperiodic conditions during the imaginal period of the parental life showed the possibility to alter the maternal influence, which determines the larval diapause in the progeny. A total of 18 combinations of photoperiods from different parts of the photoperiodic scale were studied. The physiological state of the flies and their progenies vary in accordance with the final photoperiod, i.e., there is no evidence of a stepwise photoperiodic response in *Calliphora*. The dependence of the larval diapause on the female age was confirmed. It was demonstrated for the first time that the configurations of the photoperiodic curves reflecting the maternal influence of the flies aged 20, 35, and 45 days are different. The role of the female photoperiodic response in the formation of the reserves of hibernating larvae in the temperate zone and the physiological mechanism of the maternal effect are discussed.

Control over the development of insects and their seasonal adaptations constitute one of the fundamental problems of insects ecology, which are of theoretical and practical interest. Various aspects of these problems have been reviewed by Danilevskii (1961), Tyshchenko (1977), Zaslavski (1984), Tauber *et al.* (1986), and Danks (1987). The decisive role played by the photoperiod and temperature, and also by the humidity, food and population density, in the control of seasonal development of insects was clearly demonstrated. In most cases, these factors affect various ontogenetic stages, which precede the diapausing one, but belong to the same generation. The cases of maternal influence on the diapause of progeny, when external factors exert their influence via a maternal organism, have been investigated to a lesser extent (Simmonds, 1948; Vinogradova, 1973; Mousseau and Dingle, 1991). Among insects with maternal determination of the physiological state of progeny, the best studied species is the blowfly *Calliphora vicina*, which is known as an excellent model object for ecological and physiological investigations (Vinogradova, 1984, 1991). It has been established experimentally that the photoperiodic conditions during development and sexual maturation of a maternal generation of the flies govern the norm of response to external conditions in a progeny and thereby determine its nondiapausing or diapausing development (Zinovjeva and

Vinogradova, 1972a, 1972b; Bogdanova and Zaslavski, 1985; Bogdanova *et al.*, 1985; Saunders, 1987; Saunders *et al.*, 1986, 1988, Nesin *et al.*, 1995). The maternally operating photoperiodism determines not only the fraction of diapausing larvae in the progeny, but also the intensity of their diapause (Vinogradova, 1974; Saunders, 1987). The influence exerted by changes in photoperiod (stepwise or gradual) and in nondiurnal light-dark cycles during the development of a maternal generation has been studied (Vinogradova, 1991). It is suggested that the maternal influence on larval diapause of the progeny in *C. vicina* includes two components. The main component is associated with photoperiodism and the additional one with the maternal age, with the main component affecting the additional component: the sign of the correlation coefficient between the female age and diapause of progeny depends on photoperiod (Vinogradova and Reznik, 2000).

This paper reports on an experimental study of the combined influence exerted by single (stepwise) changes in the photoperiodic regimen during the imaginal period of life of *C. vicina* flies and by their age on the larval diapause in progeny.

MATERIALS AND METHODS

The laboratory strain of *C. vicina* from St. Petersburg was used in all experiments. The method of fly

rearing has been described in detail earlier (Zinovjeva and Vinogradova, 1972). Experiments were carried out at the Laboratory of experimental entomology of the Zoological institute Russian Academy of Sciences, in thermostated boxes with automatically controlled photoperiods and temperature (fluctuations of temperature did not exceed $\pm 0.5^{\circ}\text{C}$).

In the experiments, larvae subjected to cold reactivation at $5-6^{\circ}\text{C}$ were used. Puparia were maintained under different photoperiodic regimens at 20°C , the emerged flies matured in the same conditions. The flies aged 21 days were transferred in other photoperiodic conditions according to experimental scheme; part of flies was maintained under the initial photoperiod as a control. The first egg rafts appeared in 11–12 days and then group rafts deposited every 3–4 days were placed in standard conditions (light : dark 12 : 12 at 12°C). Constant photoperiods are designated as L : D, where L and D are the numbers of hours of light and dark per day.

A single-step change in photoperiod is designated as $L_1 \rightarrow L_2$ (e.g., “12→14,” where 12 is the photophase duration in the initial regimen (in hours), and 14 that in the final regimen).

The percentage of the diapausing larvae was counted in 45 days, because this time is the optimal for the differentiation between diapausing and nondiapausing larvae. The dynamics of variation of the incidence of diapausing larvae in the progeny of flies of different ages served as a complex index, which characterized age changes and changes in the female organism in response to a new photoperiod.

The experiments included three series, with day length increased in the 1st series and decreased in the 2nd, and flies of the 2nd generation used in the 3rd series.

In each experimental variant, the number of group rafts varied from 7 to 24. The total number of rafts was 337 (78 502 larvae). Before statistic processing, the incidences of diapause were transformed for normalization, according to a formula $Y = 2 \cdot \arcsin(\sqrt{X})$ and were used for two-factor dispersion analysis. SYSTAT software was used for transformation and statistical processing of the data obtained. The initial (nontransformed) data are presented in figures with the egg rafts deposited in 10-day intervals combined (on the vertical axis, the midpoints of the corresponding intervals are designated).

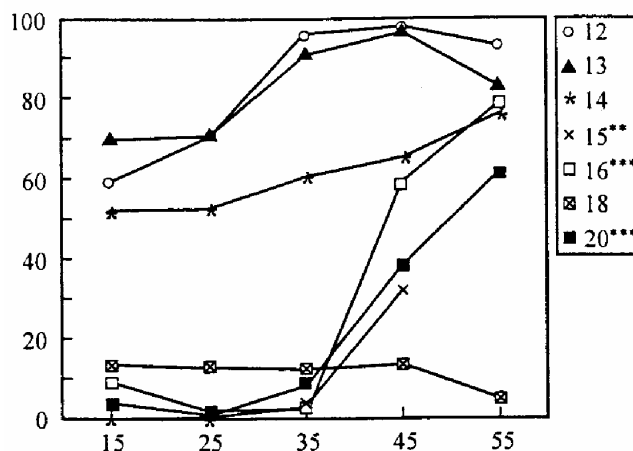


Fig. 1. Influence of the age of females maintained under different constant photoperiods on the induction of larval diapause in the progeny of *Calliphora vicina* R.-D. Abscissa: female age (days), ordinate: incidence of diapausing larvae in progeny (%). Each line corresponds to one photoperiod, the day length is showed to the right of the figure. The significance of the effect of female age on the incidence of diapause in progeny at each photoperiod is shown to the right of the day length (ANOVA test): * $p < 0.01$, ** $p < 0.001$.

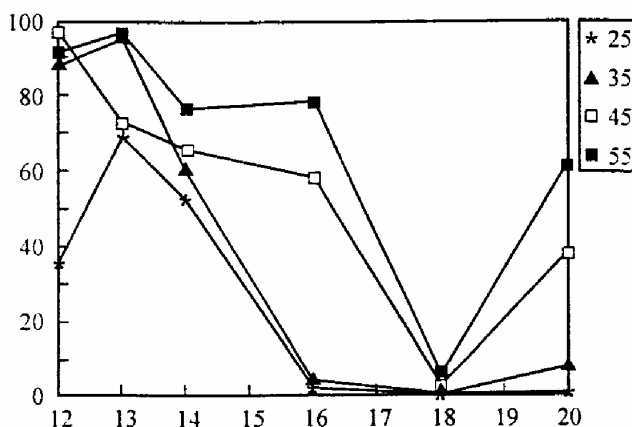


Fig. 2. Influence of the photoperiodic regimen, in which the females were maintained, on the induction of larval diapause in the progeny of *Calliphora vicina* R.-D. Abscissa: day length (hours), ordinate: the same as in Fig. 1. Each line is a photoperiodic curve reflecting the response of progeny of females from a certain age group (the female age is shown to the right of the figure).

RESULTS

Prior to considering the response of flies and their progeny to changes in photoperiodic regimen, the response of the geographic strain of *C. vicina* under study to constant photoperiods should be analyzed (Fig. 1). It can be well seen from the response of the control insects that the constant photoperiods L : D 20 : 4, 18 : 6 and 15 : 9 are perceived by flies as a long day forcing the progeny to develop mainly without diapause, whereas the rest of the photoperiods are perceived as a short day, inducing diapause in most of

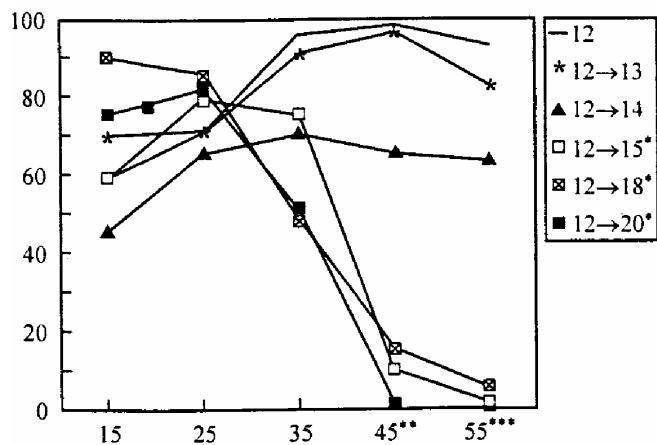


Fig. 3. Influence of the female age and a single stepwise change of photoperiod on the induction of larval diapause in the progeny of *Calliphora vicina* R.-D. (initial photoperiod is L : D 12 : 12). Abscissa: female age (days), ordinate: the incidence of diapausing larvae (%). Each line corresponds to one variant of constant or changed photoperiodic regimen. The photoperiodic regimens are shown to the right of figure. The significance of the effect of female age on the incidence of diapause in progeny is shown to the right of the day length. The significance of the influence of the final photoperiod on the incidence of diapausing larvae in progeny produced by females of definite age group is shown near the female age (abscissa) (ANOVA test): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

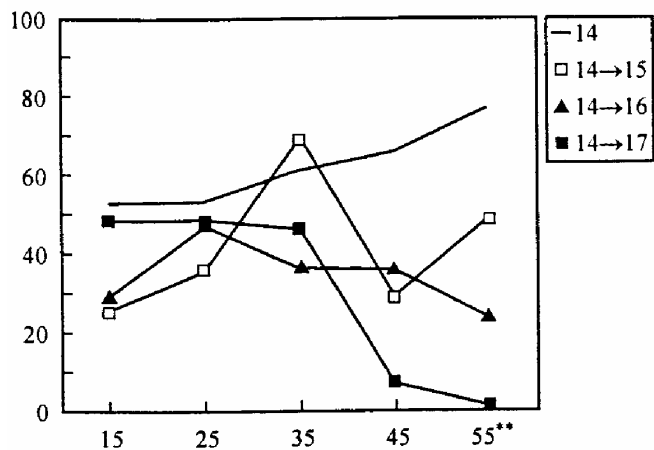


Fig. 4. Influence of the female age and a single stepwise change of photoperiod on the induction of the larval diapause in the progeny in *Calliphora vicina* R.-D. (initial photoperiod is L : D 14 : 10). For designations, see Fig. 3.

larvae; L : D 12 : 12 and 14 : 10 differ, this effect being more pronounced in the former case. The most clear difference between long and short days appears in the progeny of flies not older than 35 days. In the progenies of older flies, a significant increase in the diapause incidence is observed (excluding L : D 18 : 6). As a result, the run of the photoperiodic curve responsible for diapause induction by flies of different ages is modified (Fig.2). Only in the progeny of flies

aged 25–35 days, a small percentage of diapause is observed under long photophases (L : D 20 : 4, 18 : 6, 16 : 8), whereas in the progeny of older flies (45–55 days), the incidence of diapause is low only under the “strongest” long photophase (L : D 18 : 6), being high under other photophases.

In the 1st series of experiments, the physiological changes in flies and the corresponding reaction of their progenies to transfer of parents from short day to a set of long day photoperiods were studied (Figs. 3, 4). In experiments “12→20,” “12→18,” and “12→15” (Fig. 3) the physiological state of the progeny changes according to the final photoperiod and leads, beginning on 45th day, to a significant decrease in percentage of diapausing larvae. Beginning at this time a significant correlation between age of females and larval diapause is observed in the regimens mentioned. In experiments “12→13” and “12→14,” neither significant differences between variants nor reliable dependence of the progeny diapause on the fly age are recorded. The fraction of diapausing larvae varies from 45% to 95%, i.e. within the norm typical of the constant photoperiods L : D 12 : 12, 13 : 11, and 14 : 10 (Fig. 1).

In experiments with increasing day length (beginning from L : D 14 : 10) (Fig. 4), the response is less clear: in all the photoperiodic regimens, the incidence of the diapausing larvae changes significantly in progenies of females of different ages, and only by the 55th day, the fraction of diapausing larvae in the variants “14→16” and “14→17” decreases significantly in accordance with the final regimen.

In the 2nd series of experiments (Figs. 5–7), the response to the single-step decrease in day length was studied. Replacing the long day L : D 16 : 8 with the short day L : D 12 : 12 and 14 : 10 leads to a fast change of the physiological state of larvae from activity to diapause, with both the final photoperiod and the female age certainly involved in this process (Fig. 5).

On replacing the long-day photoperiod (L : D 18 : 6) with shorter-day photoperiod (Fig. 6), the change of the physiological state of the progeny occurs in accordance with the final photoperiod: under constant photoperiod of L : D 18 : 4, flies continue to produce nondiapausing progeny; however, after the changes “18→14” and “18→12” the incidence of diapausing larvae increases sharply beginning on 35th day.

Further, we consider the reaction of flies to a change from L : D 20 : 4 to also long-day photoperiods (“20→18,” “20→17,” and “20→15”) (Fig. 7).

In the initial regimen, flies produce almost exclusively nondiapausing progenies; however, after a change of the photoperiod the larval development is little affected; further increase in the incidence of diapausing larvae upon "20→17" is probably associated with female aging, because the same changes also occur under constant photoperiod of L : D 20 : 4. Probably the same factor is responsible for the increase in the fraction of diapausing larvae in the final regimen "20→15."

On replacing the long-day photoperiod (L : D 20 : 4) with short-day regimens (L : D 12 : 12) and 14 : 10), the physiological state of the progeny significantly changes. In two weeks, the percentage of diapausing progeny drastically increases, and by the 45th day reaches 100%.

In the 3rd series with the 2nd fly generation (Fig. 8), the result of "18→12" is the same as that in the similar experiment from the 2nd series: nondiapausing progeny is replaced by the 35th day by exclusively diapausing progeny in accordance with the final photoperiod.

DISCUSSION

It is well known that the photoperiod and temperature are the most important among the ecological factors determining the maternal effect on the diapause in insects (Vinogradova, 1973, 1991; Mousseau and Dingle, 1991). In *Calliphora*, both a gradual (daily) change in day length and a single stepwise change in photoperiod are effective (Vinogradova, 1976). If the flies were kept under short photoperiod during the first 20 days and then under long photoperiod ("12→20"), 50–60 days are necessary to change the maternal influence and, accordingly, the physiological state of the progeny; the results of a reverse change of the photoperiods are displayed earlier, after 10–15 days (Belomorsk and St. Petersburg populations) or 45 days (Nizhnii Novgorod population). Consequently, there exist certain interpopulation differences in the response to a change in the photoperiod. A significant individual variability of the time of response of some females (Nizhnii Novgorod population) to the photoperiodic change has also been revealed: from 9 to 24 photoperiodic cycles (Vinogradova and Zinovjeva, 1972).

In the present study, the number of photoperiodic combinations replacing one another during the imaginal period of the fly life was much greater, with photoperiods from different parts of the photoperiodic scale combined. In the case of combination of photoperiods

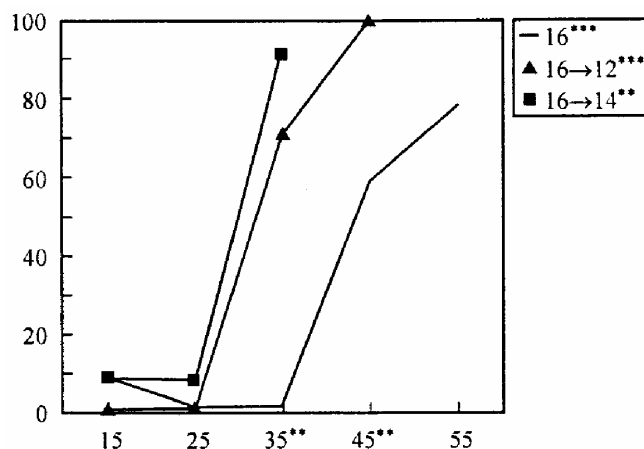


Fig. 5. Influence of the female age and a single stepwise change of photoperiod on the induction of the larval diapause of progeny in *Calliphora vicina* R.-D. (initial photoperiod is L : D 16 : 8). For designations, see Fig. 3.

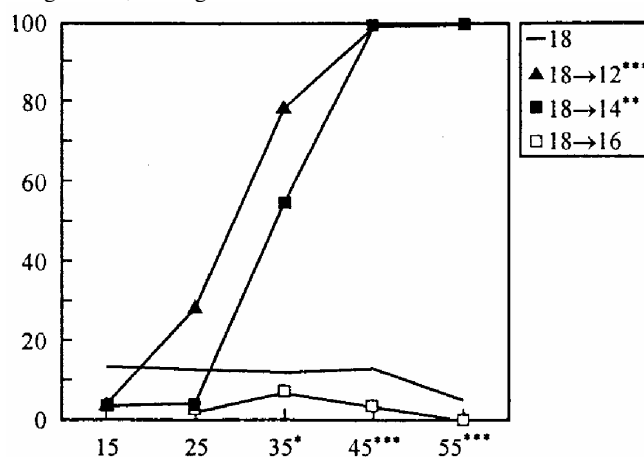


Fig. 6. Influence of the female age and a single stepwise change of photoperiod on the induction of the larval diapause in the progeny in *Calliphora vicina* R.-D. (initial photoperiod L : D 18 : 6). For designations, see Fig. 3.

inducing different percentages of diapause, the physiological state of the progeny changes in accordance with the final photoperiod. In variants with decreasing day length ("20→12", "20→14", "20→15", "18→12", "18→14", "16→14", "16→12"), females begin to produce a diapausing progeny after 15 or 25 days. In variants with increasing day length ("12→15", "12→18", "14→17"), exclusively diapausing larvae appear somewhat later, when the female age is 45–55 days. A change is usually manifested in a gradual variation of the incidence of diapausing larvae. In the case when photoperiods determining about the same inclination to diapause are combined ("20→18", "18→16", "12→13"), the pattern of progeny development does not change fundamentally, though considerable fluctuations in the percentage of diapausing individuals are observed. Such fluctuations are the

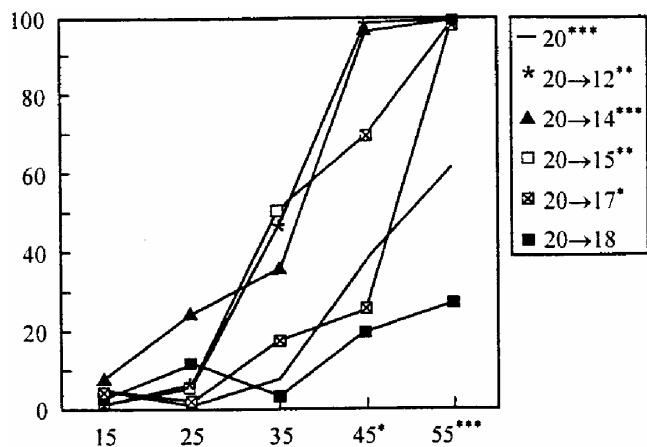


Fig. 7. Influence of the female age and a single stepwise change of photoperiod on the induction of the larval diapause in the progeny in *Calliphora vicina* R.-D. (initial photoperiod is L : D 20 : 4). For designations, see Fig. 3.

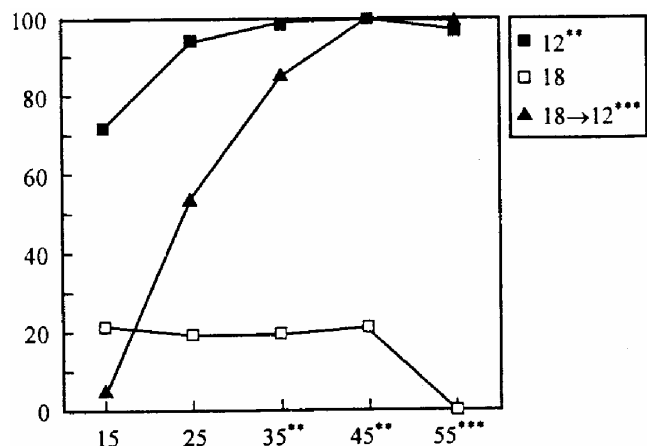


Fig. 8. Influence of the female age and a single stepwise change of photoperiod on the induction of the larval diapause in the progeny in *Calliphora vicina* R.-D. (data for the 2nd generation). For designations, see Fig. 3.

most pronounced in the variants with near-threshold photoperiods ("14→15," "14→16"). On the whole, the experiments conducted demonstrate that there is no stepwise photoperiodic reaction in *C. vicina*.

The influence of the parental age on the progeny diapause may be manifested variously: the fraction of diapausing progeny may increase with female aging, the opposite tendency and also irregular fluctuation of the percentage of diapause are also possible (Vinogradova, 1973; Mousseau and Dingle, 1991). Before this study, the relationship between the larval diapause and the parental age was revealed in four geographic strains of *Calliphora*. In the strain from England, the incidence of larvae diapausing at 11°C increased gradually at L : D 12 : 12 at temperatures of 18–24°C

and reached 100% in 11–15 days (Saunders, 1987). In two other geographic strains (Nizhnii Novgorod and St. Petersburg), the high inclination to diapause appeared only in progeny of the flies aged 40 days or more (Vinogradova, 1991). In *C. vicina* from Stavropol, a significant positive correlation between the progeny diapause and the age of flies from the long-day regimen and a negative correlation for flies kept under short-day regimens was observed (Vinogradova and Reznik, 2000). In the present study, a significant effect of the female age on the larval diapause was manifested in most of constant photoperiods. The long life of flies made it possible to obtain in constant photoperiods three different photoperiodic curves, which reflected the maternal influence of flies aged 25, 35, and 55 days. Apparently, this phenomenon is to be taken into account in similar experiments with flies, and possibly with other insects, too.

The experiments conducted made it obvious that both the components: maternally operating exogenous (photoperiodic) and endogenous (female age), are involved simultaneously in the formation of the maternal effect on progenial diapause in *C. vicina*, and special experiments are necessary to elucidate the role of each of them.

It is known that *C. vicina* can overwinter in the temperate zone in the state of reproductive and (or) larval diapause (Vinogradova, 1991). Obviously, the female takes part by means of maternal influence in the formation of a reserve of hibernating larvae. In autumn, the synchronous shortening of the day length and low temperature, which act both upon parents and upon their progeny, promotes the maximum development of the potential capacity of larvae for diapause. The long life of flies, combined with their capacity for physiological changes, suggests that the reserve of hibernating larvae may be formed by progenies of females belonging to different generations.

The physiological mechanism of the maternal influence in insects has long attracted the attention of researchers, however, the hormone of diapause, which is secreted by suboesophageal ganglion and determines the appearance of diapausing eggs, has been identified only in *Bombyx mori* (Hasegawa, 1957). Studies aimed to elucidate the nature of the factor responsible for the maternal influence have long been carried out with *Sarcophaga bullata*. In this fly, the maternal effect prevents occurrence of pupal diapause in the progeny of flies reared under short day (Henrich and Denlinger, 1982; Rockey *et al.*, 1989; Webb and Denlinger,

1998). Special experiments with different physical and chemical agents showed that high- and low-temperature shocks, starvation, and small size of females cannot change the maternal effect. However, injection of GABA (gamma-aminobutyric acid belonging to inhibitor neurotransmitters of insects) and its antagonists, picrotoxin or octopamin, induces in females the opposite effects. GABA makes lower the percentage of diapausing pupae, whereas picrotoxin increases it somewhat. The nature of maternal determinant relating two generations remains unknown. The experiments conducted showed that critical information is transmitted from the maternal brain to ovaries after pupariation but before the 2nd day of adult life, i.e. long before egg maturation. The fact that pharmacological agents can change the maternal effect inspires hope that the mechanism of transmission of the maternal information will be elucidated in the near future.

CONCLUSION

Experiments with single stepwise change of photoperiodic conditions during the imaginal period of the parental life included 18 combinations of photoperiods from different parts of the photoperiodic scale. The possibility of changing the maternal influence, manifested in that the proportion of diapausing and developing larvae in the progeny changes, was shown. The change of the physiological state of flies corresponds to the final photoperiod, no evidence of a stepwise photoperiodic response was observed in *C. vicina*.

It has been demonstrated for the first time that configurations of the photoperiodic curves reflecting the maternal influence of flies aged 20, 35 and 45 days are different.

ACKNOWLEDGMENTS

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