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Larval Diapause in *Chymomyza costata* (Diptera: Drosophilidae) I. Effects of Temperature and Photoperiod on the Development[1]

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Abstract *Chymomyza costata* is one of few drosophilid species known to commit larval diapause. The thermo-photoperiodic induction of diapause is released by rearing conditions of 18°C/LD 14:10 or shorter photocycles. At a high temperature, 23°C, diapause is not induced even in a short photocycle, LD 10:14. The stage sensitive to photoperiodic stimuli for induction of diapause lies at 18°C between 10 to 15～20 days after being oviposited.

Introduction

According to the phenological records of Drosophilidae hitherto accumulated, adult diapause is prevailing in the Holoarctic region. Pupal diapause is known only in *Drosophila alpina* (11, 12), and larval diapause in a few species, *Chymomyza amoena* (1), *Chy. distincta* (5), *Chy. costata* (7), and *Scaptodrosophila deflexa* (2). Consequently, the studies on larval diapause have less advanced than those on adult diapause. In Finnish strains the mechanisms controlling larval diapause of *Chy. costata* was reported as entirely temperature-dependent involving some geographical variation but with no reference to the role of photoperiodism (7). Recently the positive effect of photoperiod on larval diapause was confirmed in some Finnish strains (15). The present paper deals with the role of photoperiod and temperature on the development of *Chy. costata* Zetterstedt in Hokkaido, northern Japan.

Materials and Methods

Chy. costata is known from northern, central and western Europe, Hungary, western Russia, Japan (7, 14) as well as Arctic America (17). The stock used for experiments are offsprings of several females collected on July 27, 1978, by net-sweeping or direct aspirating at stumps in recently cut-overed areas and timberyards nearby Jozankei, a mountaneous suburb of Sapporo. The parental flies were allowed to oviposit on the culture medium (sugar 50, agar 14, yeast 80, water 1000) in a milk bottle kept at room temperature. Then eggs were daily collected and sterilized with 70%

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alcohol for about five minutes to prevent mold. Sixty eggs were put in each glass vial (3 cm diameter, 10 cm height) with the culture medium (10 ml), cotton-plugged, and kept in eight different thermo-photoperiodic conditions; 18±1°C/LD 8 : 16, 10 : 14, 12 : 12, 14 : 10, 16 : 8 and 18 : 6, 23±1°C/LD 10 : 14 and 16 : 8. The light cycles were administered by white fluorescent tubes. In each condition, their developmental stage was identified every two days under a dissecting microscope. The eggs remained unhatched on 2nd day (23°C) and 4th day (18°C) were excluded from the count as considered unfertilized. The identification of larval instar was made by measuring the length of mandibular hooks. In each measurement all individuals (30～60; variable by survival ratio) in one culture vial were examined and abandoned afterward to avoid the invasion of mold, which was the main cause making successive observations of larval growth difficult.

Another series of experiment was set up in order to elucidate the period sensitive to the photoperiodic stimuli inducing diapause. Under 18°C the immatures were transferred from longer (LL) to shorter (LD 10 : 14) photophase and *vice versa* at 5, 10, 15, 20, 25 and 30 (only from shorter to longer) days since being oviposited. The number of pupated individuals was daily counted in each condition. The count was continued at least for 50 days.

Further, the ovarian development of flies in various photocycles, the criterion hitherto employed to judge the incidence or not of reproductive diapause, was checked as follows: Until emergence the immatures were reared at 18±1°C/LD 16 : 8. On the first day of adult emergence, the flies were separated into eight groups and each incubated under the following

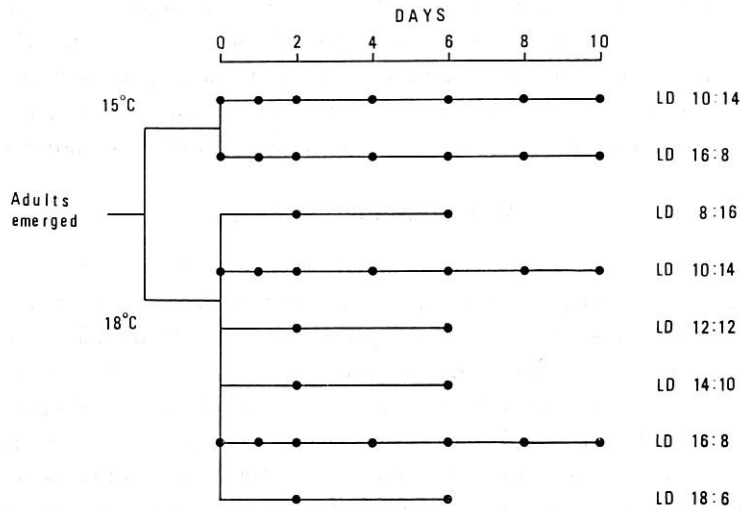

Fig. 1. The dissection schedule of adult females in various thermo-photoperiodic conditions. Adults were transferred from the stock condition, 18±1°C LD 16 : 8, soon after emergence. About 30 females were dissected on each examining date (black circle).

conditions ; 15±1°C/LD 10 : 14 and 16 : 8, 18±1°C/LD 8 : 16, 10 : 14, 12 : 12, 14 : 10, 16 : 8 and 18 : 6. About 30 individuals were reared in each glass vial (3 cm diameter, 10 cm height) containing culture medium (5 ml) and females were dissected to classify their ovarian conditions (20) according to the schedule shown in Fig. 1. About 30 females were examined at each dissection.

Results and Discussion

Figure 2 shows the effects of photoperiod on the development of immatures at 18°C. Eggs, the first instar and the second instar larvae showed no or little difference in their growth rate among the six photoperiodical cycles adopted. All eggs hatched by 4 days, almost all first instar larvae moulted by 8 days and nearly all second instar larvae moulted by 16 days after being oviposited. The third instar larvae, however, showed a significant prolongation of larval stage at LD 14 : 10 and shorter photoperiodic regimes. Larvae, having committed the developmental suppress in shorter photophases, did not begin the pupation at least by 50 days under the same photoperiodic condition. Especially in LD 10 : 14 the developmental suppress succeeded at least for 120 days. At longer photophases, LD 16 : 8 and 18 : 6, the third instar larvae began to pupate by 18 days and 26 days, respectively. But the onset and frequency of pupation were quite variable among culture vials even at the same photoperiodic condition. Adult emergence began by about 40 days in both photophases, though neither the onset nor the frequency showed synchronization among culture vials and individuals within a culture vial.

Figure 3 shows the development of the immatures both in longer (LD 16 : 8) and shorter (LD 10 : 14) photophases at 23°C. No interruption of growth was detected in either longer or shorter photophases, though the frequency of the pupation was lower in the latter.

Thus the experimental induction of developmental suppress or diapause at the third larval instar was easily realized by the artificial photoperiodic cycles LD 14 : 10 or shorter under 18°C. Under longer photophases, though larvae did not inevitably interrupt further development, timing of the initiation of pupation was quite irregular. This was probably due to other environmental factors such as humidity or undetectable invasion in culture medium of microorganisms, either harmful or nutritive for larval growth. Although the malt medium was reported to serve good nutrition for rearing this species in Finland (10), the preliminary experiment by the Japanese strain did not yield better result. Thus the other factors, which were not considered in this study also seemed to affect the diapause incidence. Such an integral influence for the induction is a characteristic feature of diapause in many insects (6, 13, 18). Nevertheless, photoperiod is considered to be in the primary factor forecasting the approach of adverse conditions in the

favourable season (16). It is well known that temperature affects the photoperiodical response of diapause or alters the critical daylength (3, 4, 8, 16). The larvae reared at high temperatures seem not to enter diapause even in short photophases. The retardation of diapause by high temperatures is also reported in several adult diapausing drosophilid species (8, 9). The

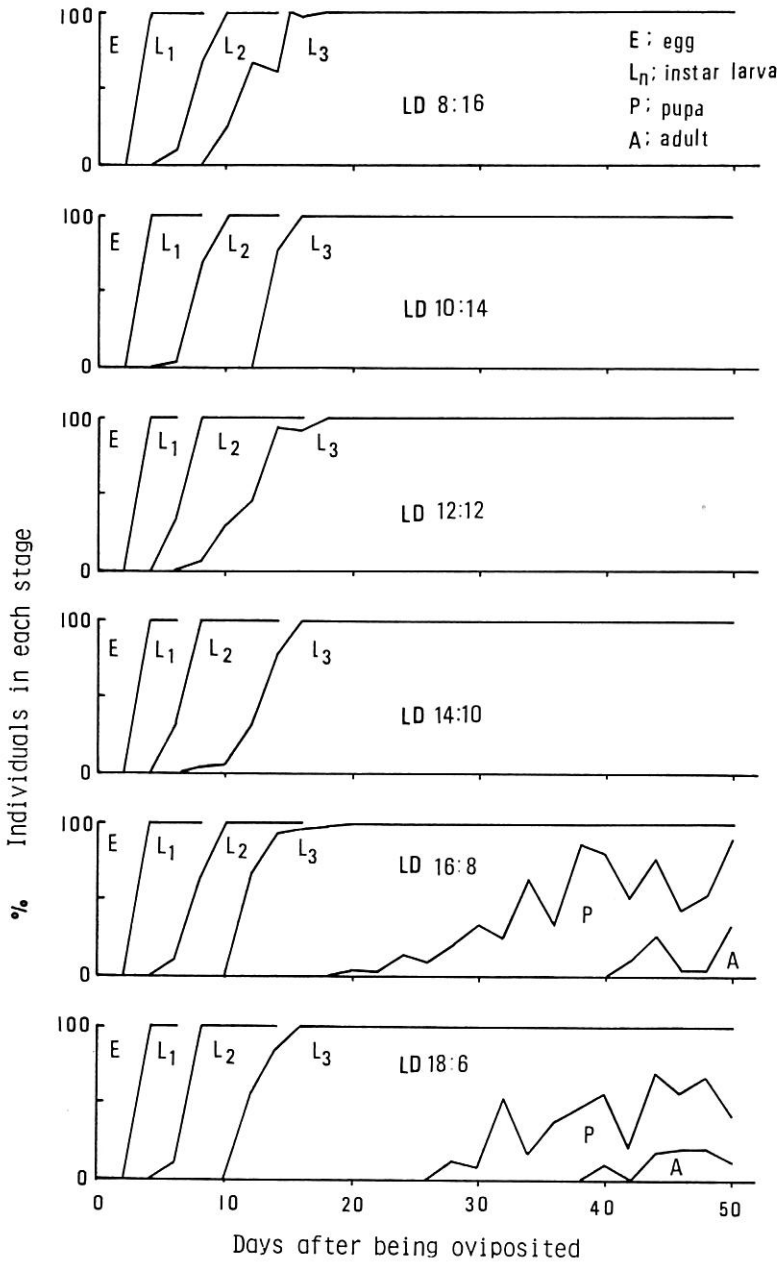

Fig. 2. Development of immatures at different photophases under 18±1°C

critical daylength lying approximately at LD 15 : 9 under 18°C and its disappearance under a high temperature, 23°C, is ecologically interpreted as follows: The natural daylength in Sapporo exceeds 15 hours a day from early June to mid July. This period seems too short to explain the adult collection records because the present data on natural population, though limited, show the production of newly emerged flies in May, June, July, August and even in early September. However, the flies emerged in May and June are considered to be overwintered individuals and therefore free from the photoperiodic condition inducing diapause. Further, the production of newly emerged flies in August and early September in spite of the shorter daylength is considered to be resulted by high temperature during July and August (monthly mean temperature; 21.2 and 21.7°C, respectively recorded by the Sapporo Meteorological Observatory). Thus the temperature compensation of the photoperiodic response seems to be significant in natural population.

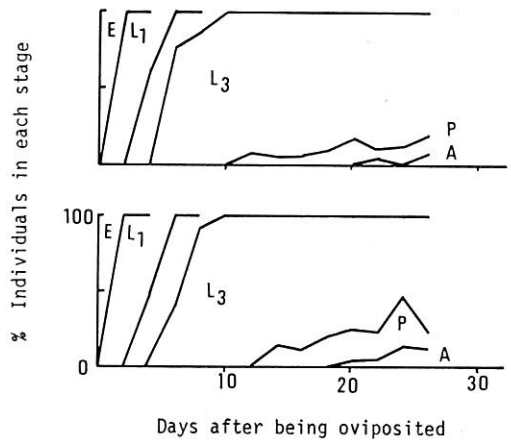

Fig. 3. Development of immatures at two different photophases under 23±1°C LD 10 : 14 (top) and 16 : 8 (bottom)

Figure 4 shows the effects of transference from a long (LL) photocycle to a short one (LD 10 : 14) or *vice versa* on the duration spent for pupation at 18°C. When larvae transferred from a shorter to a longer photoperiodic condition at 5 and 10 days after being oviposited, the mean duration of entire egg and larval stages did not significantly differ from that of LL. The larvae, transferred from a shorter regime to longer one at 15, 20, 25 and 30 days, differed significantly in their larval duration from nondiapausing individuals. While individuals transferred from LL to LD 10 : 14 at 5 and 10 days after being oviposited seemed to enter the larval diapause, the larvae transferred at 15. 20 and 25 days seemed not to be committed the developmental suppress. The transference experiment shows that first ten days, including egg, the first instar and a part of the second instar, are insensitive both to long and short photocycles. After 15 days exposure to longer photophases, larvae became also insensitive to shorter photophases, mostly completing pupation, though with a slight delay. Further, in the larvae spent their first life span in a shorter photophase for more than 15 days, the pupation after exposure to a longer photophase began with gradual delays according to the duration kept in the shorter photophase. Thus it is concluded that even after 15 days the larvae are sensitive to the shorter photophase for diapause maintenance or increasing the diapause intensity.

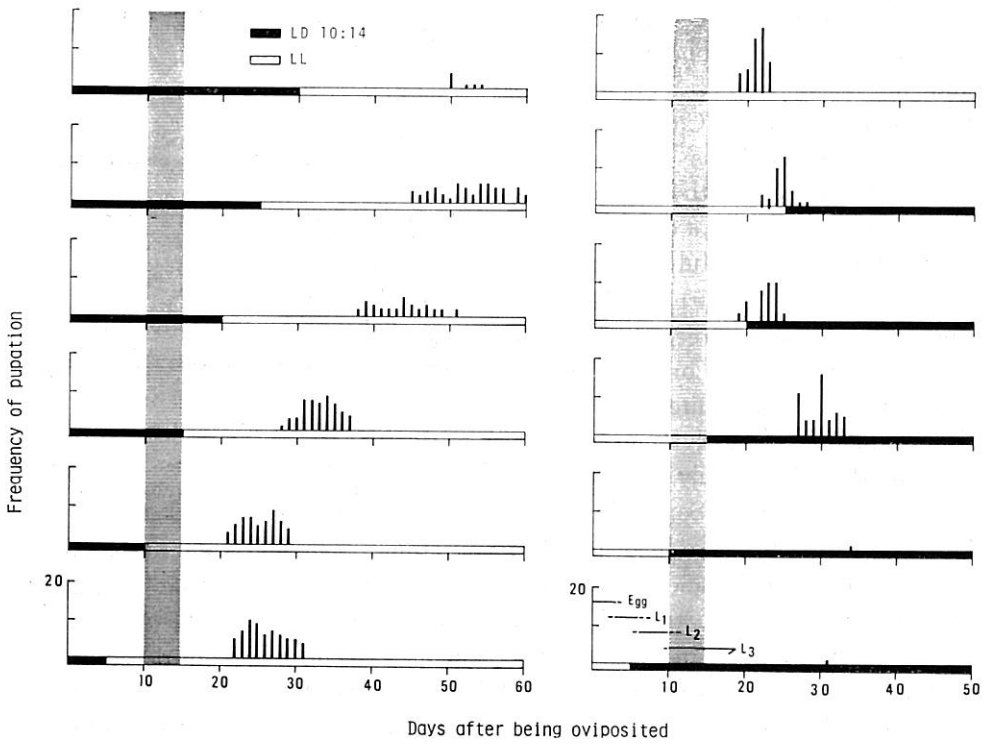

Fig. 4. Effects of transference from short (LD 10 : 14) to long (LL) photocycle or *vice versa* on the duration by the pupation. Vertical bars shows the number of pupation on each day. Shaded area indicates the photosensitive stage.

In other words, the main sensitive stage to short photocycles inducing diapause is laid after 10 days, which corresponds to the latter part of the second and the early one of the third instar. After 15 days the larvae are still sensitive to the cumulated effect of the photocycles that enhances the maintenance or intensity of diapause, though they are no more capable to enter in diapause.

Figure 5 shows the percentage of undeveloped ovaries in females dissected about 30 for each experiment. Under 18°C and 15°C the ovarian development proceeded without interruption both in two photoperiodic regimes LD 10 : 14 and 16 : 8. Most ovaries matured at least within 4 days after emergence at 18°C and 10 days at 15°C. The duration spent for ovarian maturation was identical between two photoperiodic regimes. Fig. 6 shows the frequency of undeveloped ovaries under various photocycles at 18°C. All photoperiodic regimes studied here were ineffective to interrupt the ovarian development. The similar inability to respond the photoperiodic stimuli in controlling the ovarian maturation was reported in several domestic *Drosophila* species in Hokkaido, hence they are regarded as incapable of outdoor overwintering (19). *Chy. costata*, therefore, should be considered

not to overwinter in the adult stage. Although precise information on the evolution of photoperiodism and diapause is not yet available for this species, the manifestation of photoperiodism in a unique developmental stage seems reasonable compared to other adult diapausing drosophilids, for it enhances the synchronization of life cycle in a population.

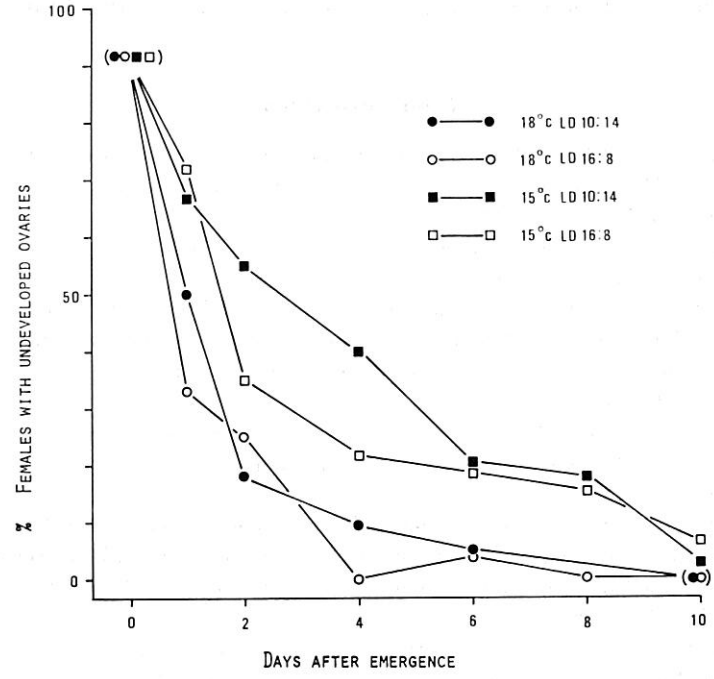

Fig. 5. Effects of temperature and photoperiod on the ovarian development. Percentage of females with undeveloped ovaries plotted as a function of time

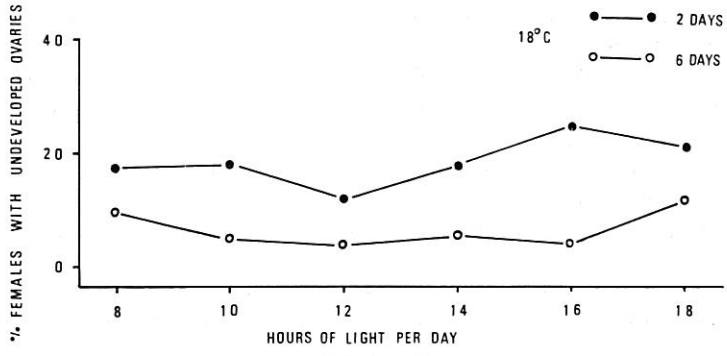

Fig. 6. Effects of photoperiod on the ovarian development in females under different photocycles at 18±1°C

Acknowledgments

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