

A mutant strain of *Chymomyza costata* (Diptera: Drosophilidae) insensitive to diapause-inducing action of photoperiod

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ABSTRACT. From a Japanese population of *Chymomyza costata* which has been known to have a photoperiodic larval diapause, we selected a mutant strain which did not respond to photoperiod. However, about 70% of the individuals of this strain entered diapause at 11°C irrespective of photoperiod, and about the same percentage of those of the photoperiod-sensitive strain also did so in continuous illumination at 11°C. This indicates that low temperature induces diapause independently of photoperiod. On the other hand, a temperature drop from 18 or 25°C to 15°C and chilling at 4°C did not induce diapause.

Key words. *Chymomyza*, larval diapause, photoperiod, temperature-dependent induction.

Introduction

In photoperiodic diapause of insects, temperature acts as a modifier of the photoperiodic response, although it may serve as the main diapause-inducing cue in some insects which inhabit equatorial regions or subterranean environments where the photoperiodic signal is neither effective nor available (for reviews see Denlinger, 1986; Tauber *et al.*, 1986). *Chymomyza costata* (Zetterstedt) occurs in Holarctic regions (Hackman *et al.*, 1970; Okada, 1976; Takada & Toda, 1981) and enters diapause at the third larval instar in response to photoperiod and temperature (Lakovaara *et al.*, 1972; Enomoto, 1981; Riihimaa, 1984).

From a Japanese population of this species, we recently obtained a mutant strain which lacked the photoperiodic response. This paper reports effects of temperature on the diapause induction in this mutant and a normal photoperiod-sensitive strain and suggests that in this species temperature can be a diapause-inducing factor independently of photoperiod.

Materials and Methods

Two laboratory strains of *Chymomyza costata* were established, one from several animals collected in the suburbs of Sapporo (43°N), Japan, in summer in 1983, and another from those collected in 1984. After collection, these strains were maintained under conditions which prevent diapause, continuous illumination and 19–23°C, for two or three generations to obtain sufficient individuals for experiments.

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The culture medium used in this study was a modification of the malt medium of Lakovaara (1969) (cornmeal 50 g, malt 90 g, agar 10 g, dry yeast 30 g, nipagin 15 ml in 1000 ml water). After arranging and cooling the culture vials, a drop of baker's yeast suspension was added to the surface of the medium.

Temperature in incubators used in the experiments fluctuated within $\pm 1^\circ\text{C}$. White fluorescent tubes (intensity of light 1000–1500 lux) were controlled by clock switches. To prevent desiccation of the medium during experiments, the culture vials were kept in polyethylene bags with small ventilation holes.

Results

Establishment of non-photoperiodic-diapause strain

Flies of the second or third generation of field-collected animals were allowed to oviposit in continuous illumination, 19–23°C for 2 or 3 days, and their eggs were placed in a short daylength, LD 11:13 h or LD 12:12 h, at 15 or 16°C. The diapause incidence (proportion of individuals which remained as larvae 45–65 days after being oviposited) of these strains was somewhat lower than 100% even at the short daylength. Individuals which did not enter diapause at the short daylength were selected and allowed to produce the next generation. One of the strains showed an

immediate response; the proportion of non-diapausing individuals at LD 12:12 h, 16°C was 16% ($n=341$) before selection, and 97.4% ($n=377$) in the next generation. The percentage of diapause remained near zero in successive generations. After the fifth generation the strain was maintained without selection. The percentage of diapause at LD 12:12 h, 15°C was still 2.9% ($n=349$) even after several generations (about a year) without selection. It was named non-photoperiodic-diapause strain (NPD).

Selection was not effective in another strain. In this strain the proportion of individuals which failed to enter diapause at LD 11:13 h, 15°C, was 9.4% ($n=244$) before selection, and 6.4% ($n=1226$) in the next generation. This strain was named photoperiodic-diapause strain (PD).

Photoperiodic response

Fig. 1 shows the photoperiodic responses of PD and NPD at 15°C. Animals were cultured at various photoperiods from the egg stage for 45–65 days and then examined for diapausing individuals persisting as larvae. NPD did not enter diapause at any daylengths, but PD showed a clear long-day type response.

Effect of temperature

Table 1 gives the effect of temperature on diapause incidence. The photoperiodic re-

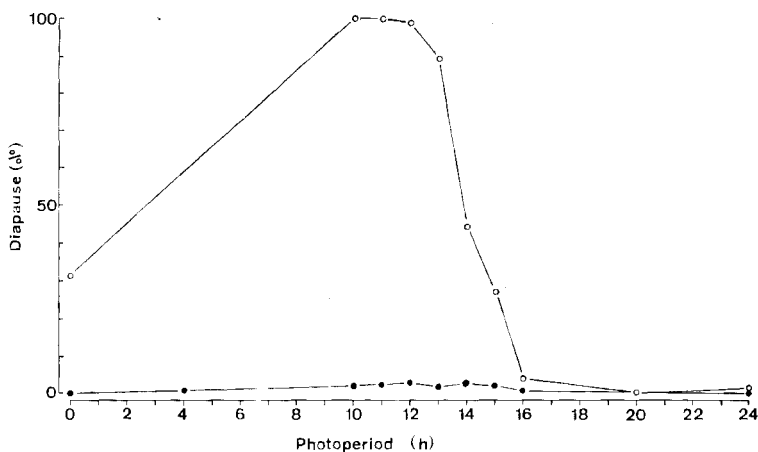


FIG. 1. Photoperiodic responses of photoperiodic-diapause (○) and non-photoperiodic-diapause (●) strains of *Chymomyza costata* at 15°C. Diapause incidence was determined 45–65 days after being oviposited. More than 100 individuals were examined for each datum point.

TABLE 1. Diapause incidence (%) of photoperiodic-diapause (PD) and non-photoperiodic-diapause (NPD) strains of *Chymomyza costata* at different temperatures in a short photoperiod (SP, LD 8:16 h at 22°C and LD 10:14 h at the other temperatures) and LL.

Temperature (°C)	Age (days after being oviposited) examined	PD		NPD	
		SP	LL	SP	LL
11	133–269	100 (43)	70.3 (111)	75.2 (106)	72.8 (67)
18	31–40	100 (102)	1.2 (83)	0 (52)	2.6 (288)
22	26–29	90.5 (178)	0 (107)	4.4 (92)	3.6 (28)
25	24–28	2.3 (177)	0 (207)	0 (33)	0 (59)

Figures in parentheses are the numbers of animals examined.

sponse of PD was clear at a temperature of 22°C or lower, but this strain did not enter diapause at 25°C even under LD 10:14 h. On the other hand, NPD did not enter diapause at a temperature of 18°C or higher, irrespective of photoperiod. However, at 11°C about 70% of individuals of PD stayed as larvae at LL (continuous illumination), and also about 70% of individuals of NPD did so irrespective of photoperiod. The remaining 30% of individuals pupariate 45–74 days after being oviposited.

To confirm that the persisting larvae at 11°C were not merely in a state of quiescence, but in diapause, they were transferred from 11°C to LL, 15°C, on the day of examination (133–269 days after being oviposited) and their development was checked 15 and 30 days later. If the larvae were in quiescence at 11°C they would soon pupariate after the transfer to 15°C, but if in diapause they would stay as larvae for a long period; these larvae did not in fact pupariate during the first 15 days, and >50% remained as larvae for >30 days (Table 2). Among these larvae, those of PD that had been reared in LD 10:14 h at 11°C were observed to be in diapause, and they pupariate after transfer to 15°C rather simultaneously with those of the

other treatment or those of NPD. Therefore, all of these larvae could be assumed to be in diapause.

For comparison, the development of post-diapause larvae of PD was examined in LL at 15°C and 11°C. The post-diapause larvae were obtained as follows. Diapause was induced at LD 11:13 h, 15°C, and was terminated by exposure to outdoor conditions from December for 1 month. The larvae were then kept at 0°C for 1 month. They were assumed to have completed diapause development and to be kept in a state of retarded development by 0°C; a temperature lower than the threshold for post-diapause development. When these post-diapause larvae were transferred to LL, 15°C, all of them ($n=72$) pupariate within 3 weeks, much earlier than the diapausing larvae transferred from 11°C (cf. Table 2). Furthermore, all post-diapause larvae ($n=117$) pupariate within 2 months at LL, 11°C. This result also suggests that the larvae that had been reared at 11°C were in diapause.

Effect of temperature-drop and chilling

Table 3 shows the effect of a temperature-drop on diapause incidence. Animals were

TABLE 2. Result of transferring larvae of photoperiodic-diapause (PD) and non-photoperiodic-diapause (NPD) strains of *Chymomyza costata* from 11°C to 15°C (LL). The cultures were examined 30 days after transfer.

Strain	Photoperiod at 11°C	No. of pupae	No. of surviving larvae	No. of dead larvae
PD	LL	40	46	25
	LD 10:14 h	15	16	12
NPD	LL	6	45	16
	LD 10:14 h	34	34	37

TABLE 3. Effect of temperature-drop in LL from 18 or 25°C to 15°C at the first, second or third instar on the diapause incidence (%) in photoperiodic-diapause (PD) and non-photoperiodic-diapause (NPD) strains of *Chymomyza costata*.

Strain	Temperature (°C) before shift	Stage of shift		
		First	Second	Third
PD	18	0 (164)	0 (163)	0.6 (164)
	25	2.9 (277)	1.2 (503)	2.9 (377)
NPD	18	1.2 (83)	1.1 (93)	1.8 (56)
	25	1.0 (81)	5.4 (180)	0 (87)

Figures in parentheses are the numbers of animals examined.

TABLE 4. Effect of chilling at 4°C (LL) on the diapause incidence (%) in photoperiodic-diapause (PD) and non-photoperiodic-diapause (NPD) strains of *Chymomyza costata*.

Strain	Period of chilling (days)	Larval stage of chilling		
		First	Second	Third
PD	8	2.3 (86)	1.7 (58)	3.7 (270)
	30	5.9 (51)	—	22.4 (170)
NPD	8	12.7 (150)	7.1 (141)	3.7 (82)

Figures in parentheses are the numbers of animals examined.

cultured at 18 or 25°C and were transferred to 15°C at the first (5 days after being oviposited at 18°C, 3 days at 25°C), second (10 days at 18°C, 6 days at 25°C), and third (15 days at 18°C, 9 days at 25°C) instar. Diapause was examined about 40 days after transfer. No effect of a temperature-drop was observed.

Table 4 shows the effect of chilling on the diapause incidence. Animals were cultured at LL, 15°C, and exposed to 4°C for 8 or 30 days at the first (8 days after being oviposited), second (16 days) or third (24 days) instar. After chilling they were transferred to LL, 15°C, and examined for diapause about 40 days after chilling. No effect of chilling was observed in most cases, but the diapause incidence was somewhat increased when third instar larvae were exposed to 4°C for 30 days.

Discussion

A strain of *Chymomyza costata* which lacked photoperiodic response was established by selection for only one generation. This selection and unpublished crossing experiments indicate that the disappearance of photoperiodic

response is expression of a single recessive gene. This might be ascribed to mutation, since we could not find this trait again in the natural population.

This mutant strain requires somewhat greater care for maintenance than a normal photoperiodic-diapause strain. Fecundity or viability of mutants may be low. A study on physiological characteristics of this mutant strain is now in progress.

Low temperature (11°C) induced larval diapause not only in the photoperiodic-diapause strain of this species but also in the non-photoperiodic-diapause mutant. This suggests that this species has a temperature-dependent induction mechanism which is effective in the absence of a photoperiodic cue. In this species, this temperature dependency may be basic for diapause induction, and photoperiod may act to modify the temperature response, i.e. the critical temperature of this temperature-dependent induction mechanism (temperature at which 50% of animals enter diapause) falls between 11 and 15°C at long daylengths, but it rises above 22°C at short daylengths when the photoperiodic response system is normal.

Although it is not known how low tempera-

ture induces diapause, a decrease in developmental rate may be important in this species. Bottella & Ménsua (1985, 1987) observed that some larvae of this species entered diapause under crowded conditions that retarded larval development. On the other hand, a temperature drop or chilling had no or little effect on diapause induction. This might be because temperature drop itself did not retard the larval development and chilling almost inhibited development.

The critical daylength of PD was about 14 h (cf. Fig. 1), somewhat shorter than that obtained by Enomoto (1981), who observed larvae persisting for a long period even at LD 16:8 h or LD 18:6 h, 18°C, but such delay of development was not observed in our study. These differences may be due to difference in culture medium: Enomoto's medium (dry yeast, sugar, agar) seemed less suitable than ours, because this species took about 35 days from egg to pupariation at long daylengths at 18°C on Enomoto's medium, but only about 20 days on our medium. The slow development on Enomoto's medium may have resulted in higher diapause rates or longer critical daylength.

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References

- Bottella, L.M. & Ménsua, J.L. (1985) Can crowding promote larval diapause in drosophilids? *Drosophila Information Service*, **61**, 39–40.
- Bottella, L.M. & Ménsua, J.L. (1987) Larval diapause induced by crowding in *Chymomyza costata* (Diptera: Drosophilidae). *Annales Entomologici Fennici*, **53**, 41–47.
- Denlinger, D.L. (1986) Dormancy in tropical insects. *Annual Review of Entomology*, **31**, 239–264.
- Enomoto, O. (1981) Larval diapause in *Chymomyza costata* (Diptera: Drosophilidae). 1. Effects of temperature and photoperiod on the development. *Low Temperature Science*, Series B, **39**, 21–29.
- Hackman, W.S., Lakovaara, S., Saura, A., Sorsa, M. & Vespäläinen, K. (1970) On the biology and karyology of *Chymomyza costata* Zetterstedt, with reference to the taxonomy and distribution of various species of *Chymomyza* (Diptera: Drosophilidae). *Annales Entomologici Fennici*, **36**, 1–9.
- Lakovaara, S. (1969) Malt as a culture medium for *Drosophila* species. *Drosophila Information Service*, **44**, 128.
- Lakovaara, S., Saura, A., Koref-Santibanez, S. & Ehrman, L. (1972) Aspects of diapause and its genetics in northern drosophilids. *Hereditas*, **70**, 89–96.
- Okada, T. (1976) Subdivision of the genus *Chymomyza* Czerny (Diptera, Drosophilidae), with description of three new species. *Kontyû, Tokyo*, **44**, 496–511.
- Riihimaa, A. (1984) The inheritance of the facultative diapause in *Chymomyza costata*. *Hereditas*, **101**, 283.
- Takada, H. & Toda, M.J. (1981) Notes on arctic Canadian Diastatidae and Drosophilidae, with the description of a new species. *Journal of Faculty of General Education, Sapporo University*, **18-A**, 1–8.
- Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York.

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