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The influence of gene flow on latitudinal clines of photoperiodic adult diapause in the *Drosophila auraria* species-complex

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Temperate species belonging to the *Drosophila auraria* species complex, *D. auraria* Peng, *D. biauraria* Bock & Wheeler, *D. triauraria* Bock & Wheeler and *D. subauraria* Kimura, enter reproductive diapause to pass the winter in response to short daylengths. These species from Japan showed latitudinal clines in critical daylength which is longer in populations from higher latitudes. The slopes of these clines coincided well with that of the cline which is approximately predicted from climatic data, suggesting that these clines result from adaptation of the species to the latitudinal gradient of climatic conditions. Between the mainlands and the surrounding islands of Japan, the slopes of clines did not differ significantly, but the deviation from the regression line was usually smaller in mainland populations. It is assumed that gene flow reduces the genetic variation among mainland populations and results in the development of smooth clines. In the plain of east China, *D. triauraria* did not show clinal variation in critical daylength, although the development of the cline is expected from climatic data. Extensive gene flow among Chinese populations is considered to prevent the development of a cline.

ADDITIONAL KEY WORDS:—Critical photoperiod – reproductive diapause.

CONTENTS

INTRODUCTION

Environmental gradients often cause organisms to develop clines in morphological or physiological traits (Endler, 1977). On the other hand, gene

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flow is expected to restrict the differentiation of discrete populations and retard the development of clines (Haldane, 1948), but such an effect of gene flow has been seldom confirmed in natural clines. In this study, we compare latitudinal clines of critical photoperiod for induction of diapause in *Drosophila* from different regions, the mainlands and surrounding small islands of Japan and the plain of east China, to evaluate the importance of gene flow in the development of clines. It is assumed that the extents of gene flow of *Drosophila* species differ among the study areas. In east China, cultivated fields spread over the plain, and mountains or forests which retard the movement of flies are present only in limited areas. In the mainlands of Japan, the topographic and environmental conditions are far more complicated: plains or open lands are divided by mountains or forests. Therefore, the level of gene flow among *Drosophila* populations is expected to be lower in the mainlands of Japan. On the other hand, *Drosophila* populations in the surrounding islands are small and almost isolated from each other and mainland populations. The study species are members of the *auraria* species complex, *D. auraria*, *D. biauraria*, *D. triauraria* and *D. subauraria*, which are distributed in temperate regions of east Asia. *Drosophila triauraria* and *D. subauraria* inhabit open lands, *D. auraria* open lands and domestic areas, and *D. biauraria* forests (Kimura, 1987). These species enter reproductive diapause to pass the winter in response to short daylengths (Minami, Kimura & Ichijo, 1979; Iwao *et al.*, 1980), and show clinal variation in critical daylength for diapause induction in the mainlands of Japan (Kimura, 1984, 1988).

MATERIALS AND METHODS

The location of collecting sites of experimental strains are shown in Fig. 1. Altitudes of these sites are lower than 100 m. Most strains of *D. auraria* were established from 10–30 field-collected females, while those of the other species were from 1–10 females. Experimental strains were maintained under continuous light at 23°C. Experiments were done within two or three generations in all strains with the exception of the strain (from site 1) of *D. auraria* and four strains (from sites 6, 13, 17 and 19) of *D. triauraria*, in which experiments were done a few years after establishment.

Insects were cultured at 15 ± 1°C from the egg stage under the following photoperiodic conditions, 10L:14D (10 h light:14 h dark), 11L:13D, 12L:12D, 13L:11D, 14L:10D, 15L:9D and 16L:8D. Diapause was examined 16 days after eclosion; females with undeveloped ovaries were recognized to be in diapause (Minami *et al.*, 1979). Usually more than 60 females were examined for each photoperiodic condition.

Prediction of the cline from climatic data

To judge whether observed clines result from adaptation of the species to the latitudinal gradients of climatic conditions, it is necessary to predict the latitudinal cline of critical daylength from climatic data. However, insufficient information is available for the accurate prediction of the cline. For this study, an approximate prediction for the latitudinal cline of critical daylength was made as follows: daylength (including civil twilight) at the date when daily mean temperature falls to 15°C in autumn was obtained for a number of

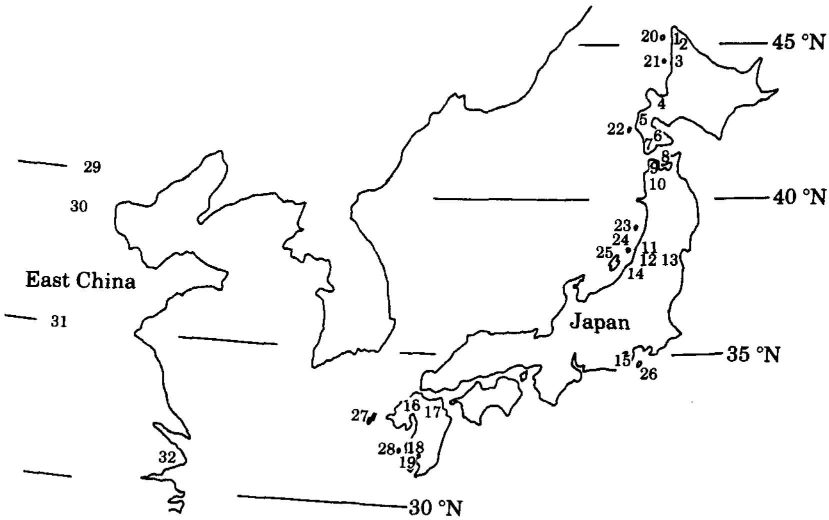

Figure 1. Location of collecting sites: the mainland (1–19) and the surrounding islands (20–28) of Japan, and east China (29–32). 1 = Wakkanai, 2 = Sarobetsu, 3 = Haboro, 4 = Sapporo, 5 = Setana, 6 = Onuma, 7 = Kikonai, 8 = Oma, 9 = Imabetsu, 10 = Futatsui, 11 = Sakata, 12 = Kiyokawa, 13 = Iwanuma, 14 = Murakami, 15 = Izu, 16 = Kashima, 17 = Oita, 18 = Sendai, 19 = Kagoshima, 20 = Rishiri, 21 = Yagishiri, 22 = Okushiri, 23 = Tobishima, 24 = Awashima, 25 = Sado, 26 = Oshima, 27 = Narao, 28 = Koshikijima, 29 = Beijing, 30 = Baoding, 31 = Heze, 32 = Shanghai. *Drosophila auraria* was collected from 23 localities in Japan and China (1, 3, 4, 5, 7, 9, 10, 11, 12, 14, 15, 16, 18, 21, 22, 23, 24, 25, 26, 27, 28, 31 and 32), *D. biauraria* was from 11 localities in northern Japan (4, 5, 7, 8, 10, 11, 14, 22, 23, 24 and 25), *D. triauraria* was from 14 localities in Japan and China (6, 7, 13, 17, 19, 22, 23, 24, 27, 28, 29, 30, 31 and 32) and *D. subauraria* was from eight localities in northern Japan (2, 4, 5, 7, 9, 20, 21 and 22).

localities and plotted against latitude (Fig. 2). This prediction is based on the observation that the induction of diapause of the study species is mainly controlled by photoperiod (Kimura, 1984) and populations of *D. auraria* and *D. biauraria* in Sapporo (No. 4 in Fig. 1) enter diapause when the daily mean temperature falls to *c.* 15°C (Iwao *et al.*, 1980).

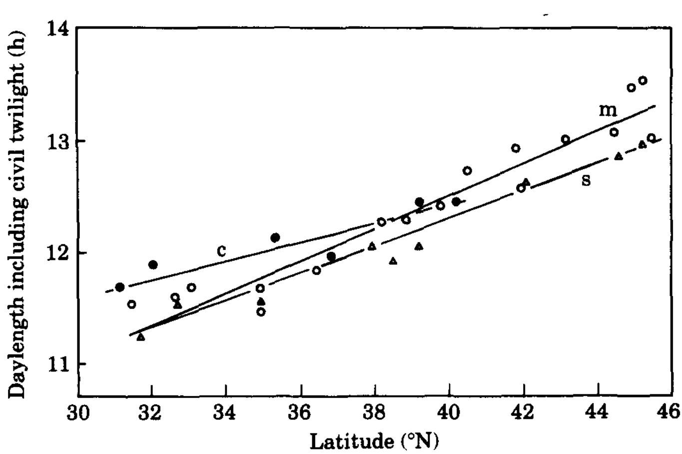

Figure 2. Latitudinal gradients of daylength (including civil twilights) at the date when daily mean temperature falls to 15°C in autumn. The mainlands of Japan (m:○), the surrounding islands of Japan (s:△) and east China (c:●).

RESULTS

Critical daylengths for diapause induction were obtained from the photoperiodic response curves by reading the 50% intercept (e.g. Kimura, 1984), and plotted against latitude (Figs 3–5). In the study species from Japan, critical daylength was usually longer in populations from higher latitudes, with regression coefficients ranging from 0.11 to 0.40 (Table 1 and Figs 3, 4). Among sympatric populations of different species, *D. auraria* always had a shorter critical daylength than the other species, but the regression coefficient (i.e. the slope of cline) did not differ significantly among these species, or between the observed clines and the predicted cline (F test, $P > 0.05$). Between the mainlands and the surrounding islands, the regression coefficient did not differ significantly (F test, $P > 0.05$), but the deviation from the regression line was larger (i.e. r^2 was smaller) in populations from the surrounding islands than in those from the mainlands except for *D. subauraria* (Table 1). An island (No. 23 in Fig. 1) population of *D. biauraria* showed a large deviation from other island populations or mainland populations (Fig. 4).

In *D. triauraria* from east China, critical daylength did not show clinal variation (Fig. 5: regression coefficient was −0.01). In *D. auraria*, the relationship between critical daylength and latitude was not clear owing to the small sample size. The difference in critical daylength between *D. auraria* and *D. triauraria* was smaller in China than in Japan.

DISCUSSION

In the mainlands and the surrounding islands of Japan, species belonging to the *Drosophila auraria* species complex showed latitudinal clines in critical daylength, longer in populations from higher latitudes. Although the clines often differed in the intercept, the slopes of clines did not differ significantly between the mainlands and the surrounding islands or among the four species. The slopes of these clines coincided well with those of clines which are approximately

TABLE 1. Regression analysis on latitudinal clines of species of the *Drosophila auraria* species complex from the mainlands and the surrounding islands of Japan

	Regression coefficient (b)	Significance of regression	Difference from $b = 0$	r^2
Predicted cline				
Mainlands	0.14	$P < 0.01$	$P < 0.01$	0.93
Surrounding islands	0.12	$P < 0.01$	$P < 0.01$	0.97
D. auraria				
Mainlands	0.15	$P < 0.01$	$P < 0.01$	0.91
Surrounding islands	0.15	$P < 0.01$	$P < 0.01$	0.77
D. biauraria				
Mainlands	0.18	$P < 0.05$	$P < 0.05$	0.76
Surrounding islands	0.15	NS	NS	0.17
D. triauraria				
Mainlands	0.11	$P < 0.05$	$P < 0.05$	0.87
Surrounding islands	0.12	NS	NS	0.51
D. subauraria				
Mainlands	0.40	NS	NS	0.71
Surrounding islands	0.26	NS	NS	0.84

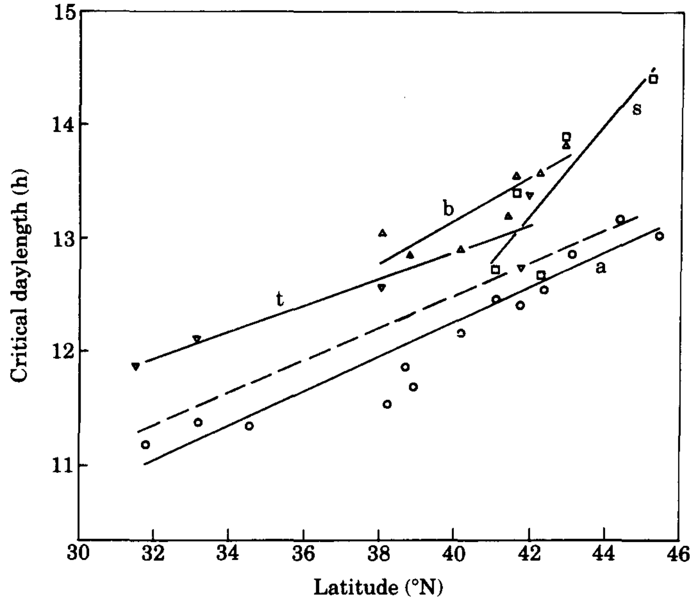

Figure 3. Relationship between critical daylength and latitude in populations from the mainlands of Japan: *D. auraria* (a:○), *D. biauraria* (b:△), *D. triauraria* (t:▽) and *D. subauraria* (s:□). Broken line shows the gradient of daylength at the date when daily mean temperature falls to 15°C (cf. Fig. 2).

predicted from climatic data. Many other insects have also been reported to show latitudinal clines of critical daylength as a result of their adaptation to the latitudinal gradient of climatic conditions (Danilevskii, 1965; Taylor & Spalding, 1986).

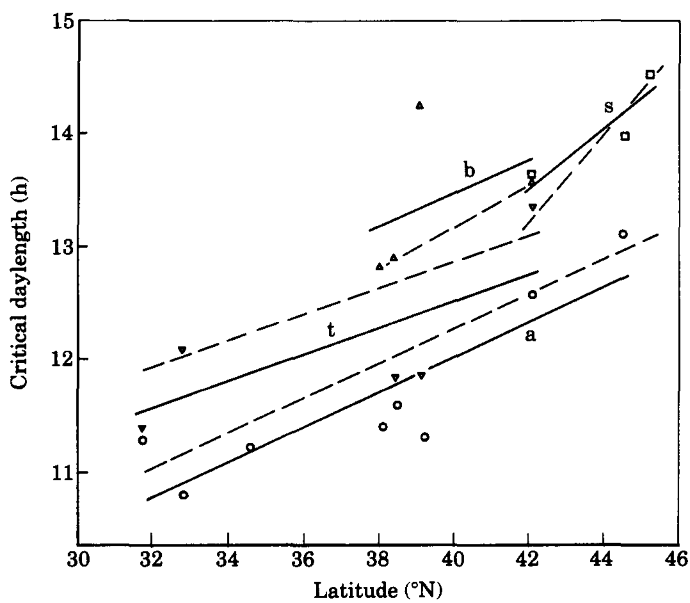

Figure 4. Relationship between critical daylength and latitude in populations from the surrounding islands of Japan: *D. auraria* (a:○), *D. biauraria* (b:△), *D. triauraria* (t:▽) and *D. subauraria* (s:□). Broken lines show the regression lines in populations from the mainlands of Japan (cf. Fig. 3).

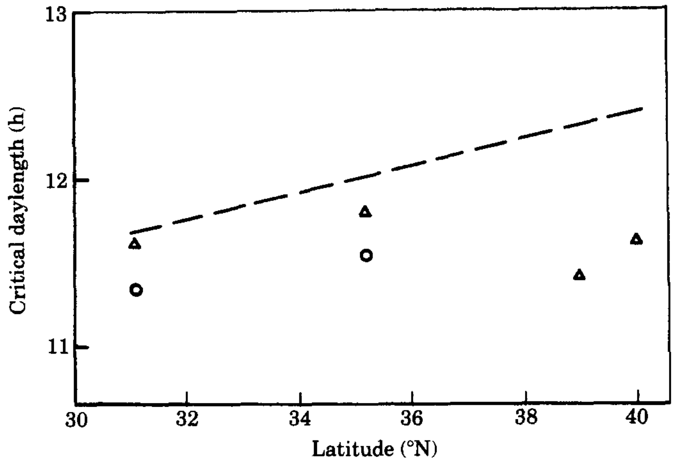

Figure 5. Relationship between critical daylength and latitude in populations from east China: *D. auraria* (○) and *D. triauraria* (△). Broken line shows the gradient of daylength at the date when daily mean temperature falls to 15°C (cf. Fig. 2).

Although the slopes of clines did not differ between the mainlands and the surrounding islands, the deviation from the regression line was usually larger in the surrounding islands. Isolated island populations may undergo genetic differentiation owing to random genetic drift or island-specific selective pressures. In the mainlands, on the other hand, gene flow is considered to reduce the genetic variation among populations and to result in the development of smooth cline. In the experimental clines of Endler (1973, 1977), gene flow also seemed to result in the development of smooth clines. It has been revealed theoretically that the amount of migration necessary to fuse local populations with respect to drift is very small (Crow & Kimura, 1970).

In the plain of east China, *D. triauraria* did not show clinal variation in critical daylength, although the development of a cline is expected from climatic data. It is assumed that extensive gene flow among the Chinese populations prevents the development of a cline. In east China, mountains or forests are present only in restricted areas, and hence drosophilid flies would sometimes be transported long distances by wind. It has been suggested that wind dispersion is important in drosophilid flies (Dobzhansky, 1973; Heed & Heed, 1972; Yoshimoto, Gressitt & Mitchell, 1962). Gene flow has also been reported to affect the development of a cline of critical daylength in Japanese populations of *D. lacertosa* Okada (Ichijô, 1986). Thus, gene flow, when it is extensive enough, counteracts selection and prevents a local population from becoming fully adapted to its environment.

Among sympatric Japanese populations of the present four species, *D. auraria* always had the shortest critical daylength; i.e. *D. auraria* enters reproductive diapause latest. Kimura (1984) assumed that the late start of diapause in *D. auraria* is a result of its adaptation to domestic environments. However, *D. auraria* and *D. triauraria* from east China did not differ so much in their critical daylengths. This may be related to the fact that adaptation of their local populations is disturbed by gene flow, or it may be that these two species do not differ in habitats in east China.

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