Cellular Basis and Developmental Timing in a Size Cline of *Drosophila melanogaster*

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Manuscript received August 9, 1994
Accepted for publication March 15, 1995

ABSTRACT

We examined 20 *Drosophila melanogaster* populations collected from a 2600-km north-south transect in Australia. In laboratory culture at constant temperature and standard larval density, a genetic cline in thorax length and wing area was found, with both traits increasing with latitude. The cline in wing area was based on clines in both cell size and cell number, but was primarily determined by changes in cell number. Body size and larval development time were not associated among populations. We discuss our results in the context of selection processes operating in natural and experimental populations.

BODY size is strongly associated with fitness in various species of animals (ROFF 1992; STEARNS 1992). In laboratory studies on *Drosophila melanogaster*, adult life history characteristics such as female fecundity (ROBERTSON 1957; TANTAWY and RAKHA 1964), adult longevity (TANTAWY and RAKHA 1964; PARTRIDGE and FOWLER 1992; but see HILLESHEIM and STEARNS 1992) and male mating success (EWING 1961, 1964) have shown positive genetic correlations with body size. In contrast, negative genetic correlations of body size with larval development rate (PARTRIDGE and FOWLER 1993), and consequently with larval competitive ability and larval viability (SANTOS *et al.* 1992; PARTRIDGE and FOWLER 1993), have been found. Thus, body size may display an intermediate optimum and be under stabilising selection, as a result of conflicting selection on the preadult and adult periods.

In natural populations of Drosophila, geographic variation in body size has been shown to be in part genetic (*e.g.*, REED and REED 1948; MCFARQUHAR and ROBERTSON 1963; DAVID *et al.* 1977; ROBERTSON 1987). Genetic body size clines have been found in various species of Drosophila and on different continents in *D. melanogaster* and *D. simulans* (STALKER and CARSON 1947, 1948; PREVOSTI 1955; MISRA and REEVE 1964; LOUIS *et al.* 1982; COYNE and BEECHAM 1987; CAPY *et al.* 1993; IMASHEVA *et al.* 1994; but see SOKOLOFF 1965, 1966); body size increases with latitude and altitude. The repeatability of these clines implies that they are caused by natural selection. Climatic factors that vary with both latitude and altitude such as temperature, rainfall and relative humidity may be involved in the selective processes shaping the clines.

The evolution of replicated laboratory populations of Drosophila in different thermal regimes has established the importance of temperature as a selective agent. Adaptation to lower temperatures has resulted in increased body size (ANDERSON 1966, 1973; PARTRIDGE *et al.* 1994a). Other studies reported similar results, but could not discriminate between the effects of natural selection and genetic drift because of lack of replication (POWELL 1974; CAVICCHI *et al.* 1985, 1989; LINTS and BOURGOIS 1987).

Adult body size could be the target of thermal selection if the fitness advantage of larger size is greater at lower temperatures. At present there is little evidence to support or refute this hypothesis. Alternatively large adult body size may be equally favored by selection at different temperatures, but lower temperatures may select for more efficient growth during the preadult period (NEAT *et al.* 1995). The plausibility of these alternatives can be tested by examining the duration of preadult development. Selection at lower temperatures in the laboratory leads to faster preadult development (ANDERSON 1966, 1973; HUEY *et al.* 1991) as a result of faster larval development (PARTRIDGE *et al.* 1994b; JAMES and PARTRIDGE 1995). These results imply that events during larval development are indeed important for the thermal evolution of body size. The pattern of genetic correlation between adult body size and rate of larval development produced by thermal selection is the opposite to that observed with artificial selection at a single temperature, where large adult size is associated with extended preadult development (ROBERTSON 1957, 1960, 1963), again as a result of an extended larval period (PARTRIDGE and FOWLER 1993).

In Drosophila, the hormonal events leading to pupariation are initiated early in the third larval instar when a critical size is reached, after which there is a fixed period of postcritical feeding and growth before pupariation occurs (BEADLE *et al.* 1938; BAKKER 1959, 1961;

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ROBERTSON 1963). Variation in larval critical size and feeding rate during the postcritical period leads to differences in adult body size (SEWELL *et al.* 1975; BURNET *et al.* 1977). Temperature throughout preadult development can affect adult body size (MASRY and ROBERTSON 1979; DAVID *et al.* 1983), suggesting that it must affect both critical weight and growth in the postcritical period. Laboratory thermal selection was shown to lead to adaptation in larval critical weight at the temperature of selection (PARTRIDGE *et al.* 1994b). Variation in adult body size in Drosophila can be a result of altered cell size or cell number. Each epidermal cell in the wing blade secretes a cuticular trichome so that the average cell size for a given region of the wing can be measured directly and used to produce an index of the total number of cells in the wing (DOBZHANSKY 1929). During development, temperature affects predominantly cell size (ROBERTSON 1959a; CAVICCHI *et al.* 1985; PARTRIDGE *et al.* 1994a), whereas food abundance and composition affect predominantly cell number (ROBERTSON 1959a). The body size divergence among populations during laboratory thermal selection is attributable primarily to changes in cell size (PARTRIDGE *et al.* 1994a), in contrast to the response to artificial selection for body size at a single temperature, which is achieved mainly through changes in cell number (ZARAPKIN 1935; ROBERTSON 1959b, 1962; L. PARTRIDGE, R. E. LANGELAN, K. FOWLER and V. FRENCH, unpublished results). Temperature is therefore unusual in changing body size through altered cell size.

In this study, we investigate a latitudinal cline in body size in Australian *D. melanogaster*. These populations show a cline in development time, with more rapid larval development at higher latitudes (JAMES and PARTRIDGE 1995). We test for a latitudinal association between rapid larval development and large adult body size, as found in laboratory thermal evolution, which would suggest that temperature is a selective agent responsible for latitudinal clines in these traits in nature. We also examine the relative importance of cell size and cell number in causing size variation in wing area. If the latitudinal clines are a consequence solely of thermal selection, we might expect the cellular basis of their altered body size to be the same as for laboratory thermal lines.

MATERIALS AND METHODS

The geographic stocks originated from flies collected at sites on a 2600-km transect along the eastern coast of Australia during February, 1993. The collections were sampled from 13 latitudes, seven of these with two replicate sites (Figure 1; Table 1). Population cages were initiated with 25 males and 25 females from each of 30 isofemale lines from each site and kept at 16.5°. The experiments described here were conducted at 16.5° and <9 mo after the flies were collected.

In the first experiment, egg collections were made on yeasted grape juice and agar plates in each population cage for an 8-hr period. As first instar larvae hatched (~48 hr after the egg lay) they were collected and transferred in lots of 50 to vials containing 7 ml of yeasted Drosophila medium. An average of 14 vials were collected per site; one site (*LH*) was eliminated because not enough eggs were obtained. Upon eclosion adults were collected and frozen. Five individuals of each sex were chosen randomly; from each, replicate vial and thorax lengths were measured with an eyepiece graticule, by placing the individuals on their right sides under a dissecting microscope at ×25 magnification. The length of the thorax was estimated to the nearest 0.02 mm from the base of the most anterior humeral bristle to the posterior tip of the scutellum.

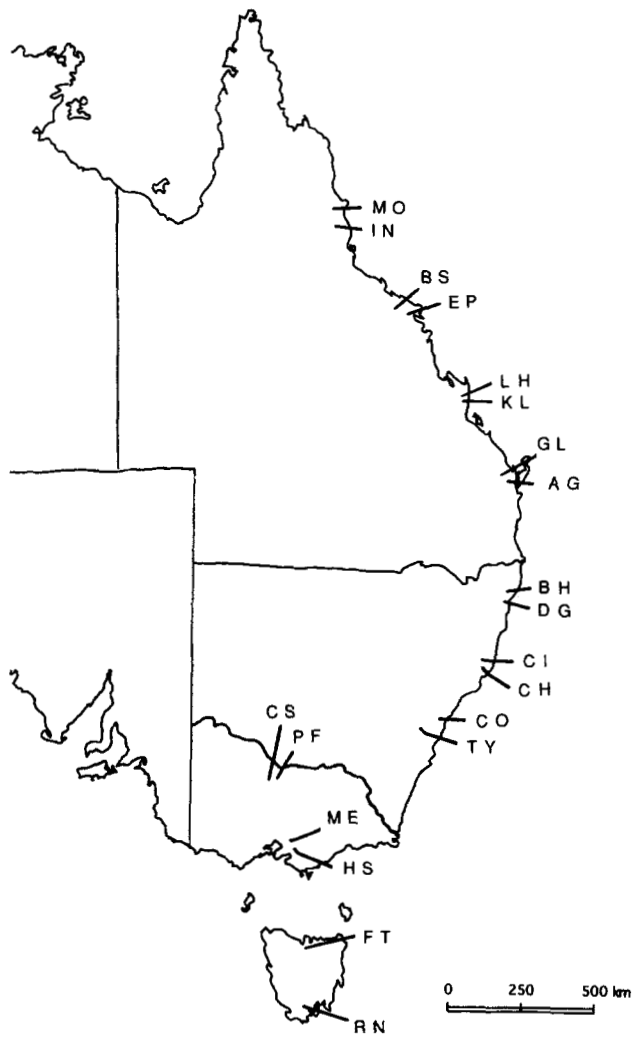

FIGURE 1.—Map of the eastern coast of Australia and the sampled populations.

In the second experiment, eggs were collected from each cage in yeasted bottles of medium. The eclosing adults were then transferred to "laying pots" containing a yeasted medium of grape juice and agar. After time for the adults to acclimate to their new environment and a prelay period to allow females to oviposit any retained eggs, the flies were given three sequential fresh laying pots at 4.5-hr intervals. Upon hatching, first instar larvae were transferred into yeasted vials of medium, 30 larvae per vial and 15 vials per site. Thorax lengths of five individuals of each sex, chosen randomly from each replicate vial, were measured, this time at ×50 magnification using a *camera lucida* attached to a dissecting microscope and a Summa Sketch II graphics tablet

TABLE 1

Name, location and latitude of each site and bait

Site	Latitude (°S)	Bait
High Falls Farm, Mossman, Queensland (MO)	16° 53′	Tropical fruit compost heap
Innisfail Banana Farm, Innisfail, Queensland (IN)	16° 53′	Banana farm dump
Big Red Fruit Stand, Bowen, Queensland (BS)	20° 01′	Banana baits around fruit stand
El Pedro Caravan Park, Bowen, Queensland (EP)	20° 01′	Banana baits
Koppel Farm, Yeppoon, Queensland (KL)	23° 08′	Pineapple and banana baits
Lazy Harry's Farm, Yeppoon, Queensland (LH)	23° 08′	Banana farm dump
Agnes' Farm, Hervey Bay, Queensland (AG)	25° 33′	Rotting mangoes
Goodlife Pools, Hervey Bay, Queensland (GL)	25° 33′	Rotting mangoes
Brunswick Heads, Northern N. S. W. (BH)	27° 57′	Banana baits
Dead Goose Farm, Norhtern N. S. W. (DG)	27° 57′	Banana baits
Coffs Harbour, Coffs Harbour, N. S. W. (CH)	30° 19′	Banana baits
Corindi, Coffs Harbour, N. S. W. (CI)	30° 19′	Banana baits
Coopernook, Taree, N. S. W. (CO)	31° 54′	Orange peels and rotting persimmons
Tyrells, Hunter Valley, N. S. W. (TY)	32° 42′	Vineyard grape peels
Cornish Farm, Cobram, Victoria (CS)	35° 49′	Squished pears
Pullars Farm, Cobram, Victoria (PF)	35° 49′	Mixed fruit dump
Chappies Farm, Melbourne, Victoria (ME)	37° 41′	Nectarine dump
Hastings Farm, Melbourne, Victoria (HS)	38° 14′	Mixed fruit dump
Forth, Tasmania (FT)	41° 11′	Fallen tomatoes
Ranelagh, Tasmania (RN)	42° 53′	Crushed apples

connected to an Apple Macintosh SE30 computer. In 12 vials per site, a wing from each of two to four flies per sex was removed, fixed in propanol, and mounted in Aquamount on a microscope slide. The areas of the mounted wings were measured on the graphics tablet by tracing their outlines starting at the humeral-coastal break. The trichomes in a standard 0.0116 mm^2 area of the same wings (in the posterior medial cell, equidistant from the fourth longitudinal vein, the posterior cross vein and the fifth longitudinal vein) were individually marked on a piece of paper, using a compound microscope at ×400 magnification with a *camera lucida* attachment, and counted. The average cell area of a wing was estimated by dividing 0.0116 mm^2 by the trichome count, and an index of the total number of cells in the wing was calculated by dividing the area of the wing by the average cell area. Although cell size varies throughout the wing, wing area is known to be determined by concordant cell size differences among distinct regions (DELCOUR and LINTS 1966; PARTRIDGE *et al.* 1995), so using an index of total cell number based on one region is legitimate.

From weather station data (GENTILLI 1971) mean wet bulb temperature at 9 AM and 3 PM both yearly and during the fly season (November through March) were calculated and correlated with latitude.

RESULTS

The means of all traits for each sex were calculated per vial in both experiments. The vial means were used to calculate the mean and 95% confidence limits for flies from each site (Figures 2 and 3).

To investigate the sources of variation in each character, we performed two-way nested analyses of variance with sex and site as crossed fixed effects and vial as a random effect nested within site. In all characters we found significant variation among sexes and sites ($P < 0.001$), and among vials within sites ($P < 0.001$) except

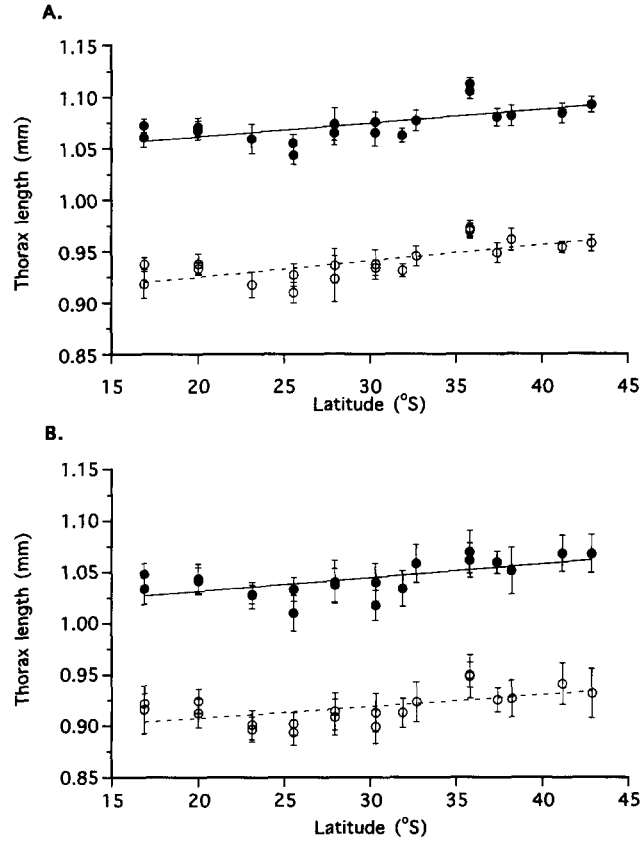

FIGURE 2.—Mean thorax length and 95% confidence intervals of flies in each site from experiment I and linear regression with latitude. (A) Experiment 1: females (●; $y = 1.0348 + 0.0013x$), males (○; $y = 0.8935 + 0.0016x$). (B) Experiment 2: females (●; $y = 1.0044 + 0.0013x$), males (○; $y = 0.8844 + 0.0012x$).

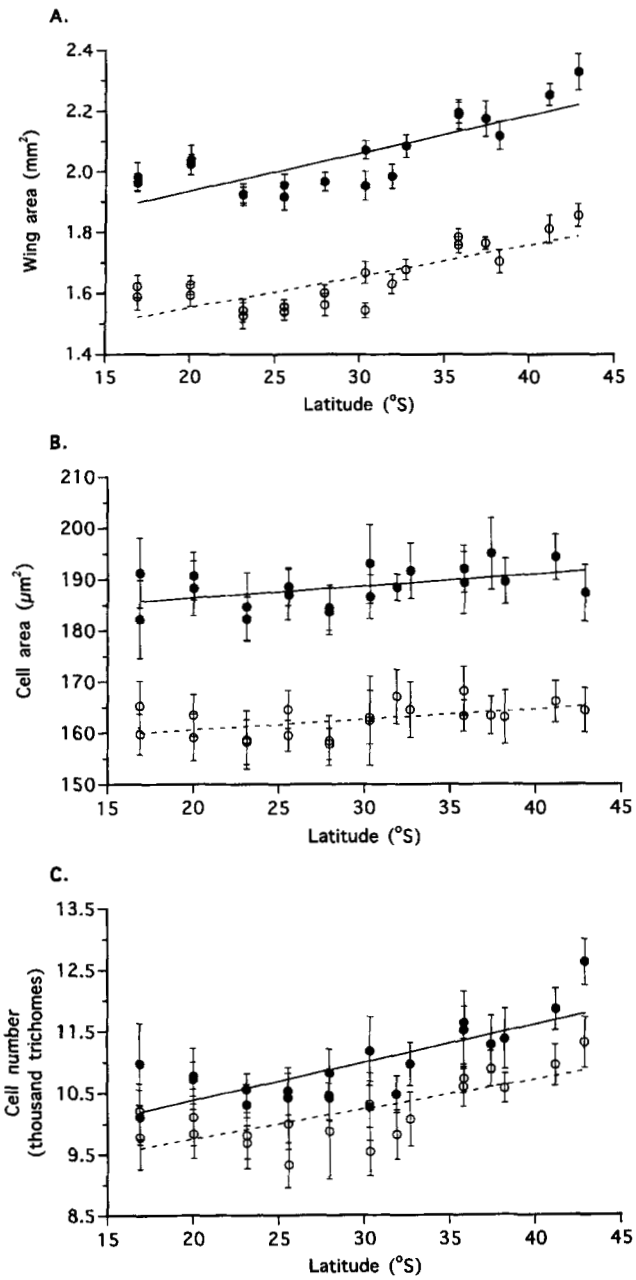

FIGURE 3.—Mean wing area and 95% confidence intervals of flies in each site and linear regression with latitude. (A) Wing area: females (●; $y = 1.6859 + 0.0125x$), males (○; $y = 1.3519 + 0.0101x$). (B) Wing cell area: females (●; $y = 181.954 + 0.227x$), males (○; $y = 156.713 + 0.200x$). (C) Wing cell number: females (●; $y = 9.1599 + 0.0612x$), males (○; $y = 8.7838 + 0.0483x$).

thorax length in experiment I ($P = 0.059$). In experiment II, significant sex by site interactions were found for thorax length ($F_{[19,275]} = 5.67$, $P < 0.001$) and wing area ($F_{[19,220]} = 4.39$, $P < 0.001$), and significant sex by vial interactions were found for cell area ($F_{[219,1333]} = 1.70$, $P < 0.001$) and cell number ($F_{[219,1333]} = 3.52$, $P < 0.001$).

Analyses of covariance with sex as a fixed main effect and latitude as the covariate were done in order to test for the existence of significant clines (SOKAL and ROHLF 1981, pp. 509–530). For all characters the intercepts for the sexes were significantly different, but the slopes did not differ significantly between the sexes (Tables 2 and 3). Significant regressions with latitude were found for every character (Tables 2 and 3). However, in cell area, the regression was significant in males ($F_{[1,18]} = 5.46$, $P = 0.031$) but not in females ($F_{[1,18]} = 3.39$, $P = 0.082$). Significant deviations from linearity were found in the regressions on latitude of thorax length and wing area (thorax length, experiment I, females: $F_{[11,7]} = 5.46$, $P = 0.015$; thorax length, experiment II, males: $F_{[1,17]} = 8.27$, $P = 0.005$; wing area females: $F_{[1,17]} = 7.37$, $P = 0.007$; wing area males: $F_{[11,7]} = 3.93$, $P = 0.040$), with size increasing more rapidly at higher latitudes. Cell size and number showed no significant deviation from linearity ($P > 0.30$). The addition of a quadratic term to the regression models significantly increased the proportion of residual sum of squares explained by the models only for wing area (females: $F_{[1,17]} = 24.2$, $P < 0.001$; males: $F_{[1,17]} = 21.2$, $P < 0.001$). The mean thorax length at each site was repeatable in the two experiments, as measured by the partial correlation between the two when latitude was held constant (females: $r = 0.677$, $P = 0.001$; males: $r = 0.792$, $P < 0.001$).

To compare the strength of the clines for the different traits in each sex, we standardized each dependent variable and latitude and estimated the standardized slopes (Table 4). All regressions were positive and the clines in wing area and cell number were steeper than the ones in thorax length and cell area, although the slopes did not differ significantly among traits.

To investigate whether the latitudinal variation in wing size was mostly explained by variation in cell size or cell number, we did a partial correlation analysis. There was a highly significant correlation between wing area and latitude (females: $r = 0.801$, $P < 0.001$; males: $r = 0.794$, $P < 0.001$). When cell area was held constant, the partial correlation of wing area on latitude was still high (females: $r = 0.759$, $P < 0.001$; males: $r = 0.720$, $P < 0.001$), but when cell number variation was removed it was significant only in males (females: $r = 0.364$, $P = 0.12$; males: $r = 0.482$, $P = 0.037$). The latitudinal cline in wing size was therefore determined mainly by variation in cell number.

The cellular basis of the variation in wing area within populations was investigated by analyses of covariance with site as a fixed effect and cell size and number as covariates. Both cell size and number were shown to contribute significantly to the variation in wing area within populations (females, cell area: $F_{[1,219]} = 23.8$, $P < 0.001$; males, cell area: $F_{[1,219]} = 15.6$, $P < 0.001$; females, cell number: $F_{[1,219]} = 6.93$, $P = 0.009$; males, cell number: $F_{[1,219]} = 19.7$, $P < 0.001$), and the slopes were not significantly different among populations ($F_{[19,200]} < 1$).

TABLE 2

Analyses of covariance on thorax length (mm) in each experiment

Source of variation	Experiment I			Experiment II		
	d.f.	MS	F	d.f.	MS	F
Sex	1	0.18003	1032.51***	1	0.15675	864.53***
Regression with latitude	1	0.00472	28.40***	1	0.00366	20.17***
Deviations from regression	35	0.00017		37	0.00018	
Differences between slopes	1	0.00003	<1 (NS)	1	0.00002	<1 (NS)
Deviations within slopes	34	0.00017		36	0.00019	
Total	37	0.00492		39	0.00429	

Sex was a fixed effect and latitude a covariate. NS, $P > 0.05$; *** $P < 0.001$.

Larval development time in these populations decreased with latitude (JAMES and PARTRIDGE 1995). However, larval development time and body size are not strongly associated among populations (Figure 4): at low latitudes development time decreases steeply with little increase in body size, whereas at higher latitudes body size increases steeply with little change in development time. Thorax length was not significantly correlated with larval development time in experiment I ($r = -0.255$, $P = 0.29$); in experiment II there was a significant negative correlation between the two characters ($r = -0.451$, $P = 0.046$), but the partial correlation with latitude held constant was nonsignificant ($r = -0.154$, $P = 0.53$).

All temperature variables were highly correlated with latitude, regardless of the time of day or seasons used to estimate them (9 AM approximate flying season, November through March, $r = -0.97250$; 9 AM whole year, $r = -0.97313$; 3 PM flying season, $r = -0.97668$; 3 PM whole year, $r = -0.95699$).

DISCUSSION

We have found a genetic cline in thorax length and wing area of *D. melanogaster* flies from higher latitudes eclosed as larger adults in experiments where temperature and larval density were controlled. This result is consistent with previous studies of genetic latitudinal clines of body size in ectotherms (PREVOSTI 1955; MISRA and REEVE 1964; ATCHLEY 1970; BRYANT 1977; BRYANT and TURNER 1978; LONSDALE and LEVINTON 1985; COYNE and BEECHAM 1987; CAPY *et al.* 1993; IMASHEVA *et al.* 1994). The repeated occurrence of these body size clines raises two important issues: what is the selective agent responsible and what is the target of selection?

Temperature decreases with latitude along the transect of collection. The evolution of larger body size in cooler climates was accompanied by an increase in larval development rate and cell size. Laboratory thermal selection produces a similar pattern in these characters (PARTRIDGE *et al.* 1994a, 1994b; JAMES and PARTRIDGE 1995), suggesting that temperature, or a causally associated variable, is indeed the relevant selective agent in nature. Latitudinal variation in temperature is associated with variation in other physical and biological factors and any of these could act as the proximate selective agent. However, the similarity between laboratory and natural populations in effects on body size and development time suggests that the proximate selective agent is the same in both.

Latitudinal variation in wing area was explained by clines in both cell size and cell number, with the latter having the predominant effect. This result could imply that natural selection in the field acts directly on adult body size because in artificial selection experiments, at a single temperature, cell number is the basis of additive genetic variation for body size within populations (ROB-

TABLE 3

Analyses of covariance on wing characters

Source of variation	d.f.	Wing area		Cell area		Cell number	
		MS	F	MS	F	MS	F
Sex	1	1.624	343.07***	6774.33	698.28***	5.66	36.69***
Regression with latitude	1	0.300	63.14***	106.44	10.97**	6.98	45.22***
Deviations from regression	37	0.005		9.70		0.15	
Differences between slopes	1	0.003	<1 (NS)	0.42	<1 (NS)	0.10	<1 (NS)
Deviations within slopes	36	0.005		9.95		0.16	
Total	39	0.054		185.63		0.47	

Sex was a fixed effect and latitude a covariate. Wing area in mm^2, cell area in μm^2, cell number in thousand trichomes. ns, $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4

Standardized slopes for each character and 95% confidence limits

Character	Coefficient	
	Females	Males
Thorax length (experiment I)	0.6199 ± 0.3872	0.6923 ± 0.3560
Thorax length (experiment II)	0.6197 ± 0.3872	0.5599 ± 0.4088
Wing area	0.8010 ± 0.2953	0.7938 ± 0.3001
Cell area	0.3981 ± 0.4525	0.4825 ± 0.4322
Cell number	0.7671 ± 0.3165	0.7199 ± 0.3424

Values are estimates ± $^1/_2$ 95% confidence intervals [or CI]

ERTSON 1959, 1962; L. PARTRIDGE, R. E. LANGELAN, K. FOWLER and V. FRENCH, unpublished results). However, the data on natural populations are not concordant with the results of laboratory thermal selection in which divergence in wing area was exclusively a consequence of changes in cell size (PARTRIDGE et al. 1994a). Another difference between the results from laboratory and natural populations was that in thermally selected lines the wing size variation within populations was predominantly based on cell number (L. PARTRIDGE, B. BARRIE, K. FOWLER and V. FRENCH, unpublished results), while our results showed cell size as well as cell number was involved. The ecological and genetic conditions in the laboratory and in the field are not strictly comparable. In particular, constant temperatures, absence of gene flow and limited time of evolution in laboratory thermal selection could account for the differences in the results.

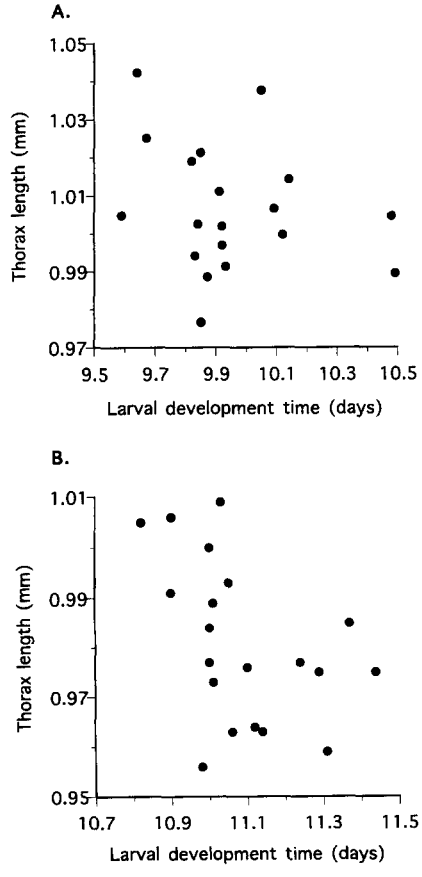

FIGURE 4.—Mean thorax length (sexes averaged) and larval development time of flies in each site. (A) Experiment I. (B) Experiment II.

It is not clear how temperature could exert an evolutionary effect on body size. Selection for desiccation resistance would produce the opposite pattern of latitudinal variation—hot climates selecting for larger body size (TANTAWY and MALLAH 1961; LEVINS 1969). Also, because small animals like Drosophila experience instant temperature changes with the environmental temperature, selection on rate of heat exchange is not likely to be important (STEVENSON 1985). The occurrence of rapid larval development at higher latitudes suggests that selection during the larval period may be important. There appears to be strong directional selection for fast larval growth rate at a single temperature (CLARKE *et al.* 1961; ROBERTSON 1963; SEWELL *et al.* 1975; BURNET *et al.* 1977; PARTRIDGE and FOWLER 1993).

Among populations body size is not associated with larval development rate, so the cline in one character can not be explained solely on the basis of a correlated response to selection on the other character. The observed patterns might be explained either by differential selection on each character at different latitudes or by selection on a third character correlated with both development time and body size causing a variable response at different latitudes. The results suggest that cooler environments may be permissive or selective of the evolution of more rapid larval growth and that this may be responsible for the evolutionary change in adult body size in response to temperature. Growth efficiency has been shown to increase under laboratory thermal selection (NEAT *et al.* 1995). It would be interesting to know if natural populations differ in other larval traits (*e.g.*, critical size, feeding rate, and growth efficiency).

The genetic basis of the observed clines could involve the segregation of the inversion *In(2L)t*, which is known to increase in frequency toward the equator in natural populations of *D. melanogaster* (INOUE *et al.* 1984). This inversion was shown to confer a survival advantage at high temperatures (VAN DELDEN and KAMPING 1989) and to slow down development and decrease body size at a range of temperatures (VAN DELDEN and KAMPING 1991). The observed latitudinal variation in body size and development time is compatible with the existence of an underlying cline in *In(2L)t*, because heterozygotes for the inversion show fast development time but intermediate body size (VAN DELDEN and KAMPING 1991).

The regression of wing size with latitude was not linear. Body size varied little between populations at lower, warmer latitudes, and the slope increased in higher, and cooler latitudes. The shape of the clines may be

caused by asymmetrical gene flow between populations along the transect. The productivity of Drosophila populations increases with temperature (BIRCH *et al.* 1963; PARTRIDGE *et al.* 1995), which could result in higher emigration rates from populations in warmer climates thus reducing the magnitude of genetic differentiation between them.

We thank ARY HOFFMANN for his kind hospitality and his logistical support and advice in the collection of the Australian lines, TRACEY CHAPMAN for assisting in collecting larvae and adults, LINDSAY LIDDLE for aid measuring thoraxes, DOROTHY CURRIE for help in computer programming, and KEVIN FOWLER and two anonymous reviewers for their useful comments on the manuscript. We thank the National Environmental Research Council TIGER program (UK), Science and Engineering Research Council (UK), the Junta Nacional de Investigação Científica e Tecnológica CIENCIA program (Portugal), the Carnegie Trust for the Universities of Scotland and the University of Edinburgh for financial support.

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Communicating editor: T. F. C. MACKAY