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Genetic and Environmental Responses to Temperature of *Drosophila melanogaster* From a Latitudinal Cline

Avis C. James, Ricardo B. R. Azevedo and Linda Partridge

Department of Biology, Galton Laboratory, University College London, London NW1 2HE, United Kingdom

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ABSTRACT

Field-collected *Drosophila melanogaster* from 19 populations in Eastern Australia were measured for body size traits, and the measurements were compared with similar ones on flies from the same populations reared under standard laboratory conditions. Wild caught flies were smaller, and latitudinal trends in size were greater. Reduced size was caused by fewer cells in the wing, and the steeper cline by greater variation in cell area. The reduction in size in field-collected flies may therefore have been caused by reduced nutrition, and the steeper cline may have been caused by an environmental response to latitudinal variation in temperature. No evidence was found for evolution of size traits in response to laboratory culture. The magnitude of phenotypic plasticity in response to temperature of development time, body size, cell size and cell number was examined for six of the populations, to test for latitudinal variation in plasticity. All characters were plastic in response to temperature. Total development time showed no significant latitudinal variation in plasticity, although larval development time showed a marginally significant effect, with most latitudinal variation at intermediate rearing temperatures. Neither thorax length nor wing size and its cellular components showed significant latitudinal variation in plasticity.

REPLICATED latitudinal clines are of evolutionary interest, because they indicate that natural selection is responsible (ENDLER 1977). They can therefore reveal both how natural selection acts on the traits and help identify mechanisms maintaining genetic variation for them. They are also important arenas in which to study the roles of genetic and environmental variation and the interactions between them in producing variation in phenotypes. In the present study we have examined the role of phenotypic plasticity in response to temperature in a latitudinal cline for development time and body size in *Drosophila melanogaster*. Our aim was to understand the role of plasticity in body size in the phenotypic expression of the cline in nature and the evolution of plasticity in the cline.

Experiments in which representatives of different populations are reared under standard laboratory conditions have revealed genetic clines in body size of *D. melanogaster* from western Europe and Africa (CAPY *et al.* 1993), eastern Europe and central Asia (IMASHEVA *et al.* 1994), North America (COYNE and BEECHAM 1987; CAPY *et al.* 1993), South America (VAN'T LAND *et al.* 1995) and Australia (JAMES *et al.* 1995), with genetically larger flies at higher latitudes (but see LONG and SINGH 1995; WORTHEN 1996). Similar size clines have been found in other Drosophila species (STALKER and CARSON 1947; PREVOSTI 1955; MISRA and REEVE 1964; DAVID and BOCQUET 1975; PEGUEROLES *et al.* 1995; but see SOKOLOFF 1965, 1966), houseflies (BRYANT 1977), honey bees (ALPATOV 1929), a copepod (LONSDALE and LEVINTON 1985b) and the Atlantic Silverside (CONOVER and PRESENT 1990) and Wood Frogs (RIHA and BERVEN 1991). Another character, larval development time, has been reported to show a latitudinal cline in *D. melanogaster* from Australia, with more rapid development in flies collected at higher latitudinal regions (JAMES and PARTRIDGE 1995). More rapid development has also been shown in populations from higher latitudes or altitudes in water striders (BLANCKENHORN and FAIRBAIRN 1995), a copepod (LONSDALE and LEVINTON 1985a,b), the Atlantic Silverside (CONOVER and PRESENT 1990, PRESENT and CONOVER 1992) and in both Green and Wood Frogs (BERVEN *et al.* 1979; BERVEN 1982; RIHA and BERVEN 1991). In *D. melanogaster*, body size and development time were not strongly associated between populations (JAMES *et al.* 1995), arguing that different genes are responsible for geographic variation in these characters.

The repeatability of latitudinal clines in different continents in *D. melanogaster* suggests that natural selection, rather than genetic drift, is responsible for their occurrence. It is likely that temperature is an important selective agent. Studies of laboratory evolution in response to constant temperatures in *D. pseudoobscura* (ANDERSON 1966, 1973) and in *D. melanogaster* (CAVICCHI *et al.* 1978, 1985; HUEY *et al.* 1991; PARTRIDGE *et al.* 1994a,b; JAMES and PARTRIDGE 1995) found that more rapid larval development to larger adult body size evolved in popula-

Corresponding author: Avis C. James, Department of Biology, Galton Laboratory, Wolfson House, University College London, 4 Stephenson Way, London NW1 2HE, United Kingdom.
E-mail: avis.james@ucl.ac.uk

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tions that were held at lower temperatures for many generations in the laboratory.

Temperature also has direct environmental effects on body size and development time in Drosophila and other ectotherms. In general, growth at lower experimental temperature results in slower development to larger adult size (ATKINSON 1994). The evolutionary and environmental effects of temperature therefore reinforce one another for body size but oppose one another for development time. The mechanisms underlying these evolutionary and environmental effects of temperature are obscure (PARTRIDGE and FRENCH 1996), although the repeatability of the evolutionary effects suggests that they are adaptive. It has not been established if the patterns of phenotypic plasticity for these traits in response to temperature are adaptive.

The persistence of latitudinal variation in *D. melanogaster* reared under standard conditions indicates that the clines seen in nature have a genetic basis. However, the contributions of genetic variation, environmental variation and gene by environment interaction to the production of phenotypic clines in nature has rarely been examined. A study of latitudinal variation in wing length in *D. melanogaster* (COYNE and BEECHAM 1987) found a steeper cline for the trait in flies reared in the laboratory at three different temperatures than in the flies collected from nature. This finding suggests that the environmental and genetic effects of latitudinal variation in temperature on body size were swamped by other sources of environmental variation. However, these populations were collected in different years and seasons, which could result in much greater environmental heterogeneity than would be present in the cline at a single time.

Plasticity of development time can change as a result of evolution at constant temperatures, with high-temperature populations showing greater plasticity (HUEY *et al.* 1991; PARTRIDGE *et al.* 1994b; JAMES and PARTRIDGE 1995), indicating both the presence of genetic variation for plasticity and natural selection for it. Phenotypic plasticity of body size in response to temperature in *D. melanogaster* is heritable (SCHEINER and LYMAN 1989) and responds to artificial selection (SCHEINER and LYMAN 1991) but did not respond to thermal selection in the laboratory (PARTRIDGE *et al.* 1994a). Variation in plasticity with latitude could contribute to latitudinal variation in these traits in nature. ETGES (1989) found significant genotype by environment interactions in egg-to-adult development time and age at first reproduction when he raised *D. robusta* from populations that had originated from different altitudes at different temperatures, but no altitudinal trends in plasticity were reported. In 10 geographic lines of *D. melanogaster* COYNE and BEECHAM (1987) found phenotypic plasticity with rearing temperature for wing length, but no latitudinal trend.

In the present study we collected adults in the field at 19 sites along the coast of eastern Australia, all in the same month of the same year (JAMES *et al.* 1995). We measured them for size traits (thorax length, wing area, cell area and cell number in the wing). Flies from these populations had previously been scored for these traits when reared under standard laboratory conditions (JAMES *et al.* 1995). Latitudinal variation in size in *D. melanogaster*, as measured in the wing and at a single temperature, is caused mainly by variation in cell number, although cell size partly contributes to the differences (JAMES *et al.* 1995). In contrast, plasticity in response to temperature is produced mainly by changes in cell size (ALPATOV 1930; ROBERTSON 1959; DELCOUR and LINTS 1966; MASRY and ROBERTSON 1979; PARTRIDGE *et al.* 1994a). Larval crowding and poor nutrition reduce size via a reduction in both cell number and cell size (ROBERTSON 1959). In the present study we compared the patterns of clinal variation in cell size and cell number in wild caught flies with those of flies from the same populations reared under standard conditions. The aim was to determine the role of environmental variation in general and of variation in temperature in particular in the phenotypic expression of the size cline in nature. To test for latitudinal variation in plasticity, we examined three pairs of populations collected at high, medium and low latitudes along the cline and examined their phenotypic plasticity in response to temperature for development time and wing area. We also measured the contributions of cell size and cell number to variation between populations in plasticity of wing size in response to temperature.

MATERIALS AND METHODS

Geographic lines: *D. melanogaster* from 20 sites over a 2600-km latitudinal transect along the east coast of Australia were collected in February 1993 and have been maintained since as population cage cultures initiated with the offspring (25 individuals per sex) of each of 30 isofemale lines from each site. The cages were initially kept at 16.5°, and after ~1 yr were transferred to 18°. The transect has been described in detail elsewhere (JAMES and PARTRIDGE 1995; JAMES *et al.* 1995).

Field collected flies: Flies collected at 19 of the field sites were preserved in alcohol [from all original sites except both sexes from a Tasmanian site (FT) and males from a Queensland site (IN), which were lost]. Measurements of thorax lengths were conducted on 25 individuals of each sex. Wing area, cell area and cell number were measured on 10 males and 10 females per site.

Laboratory adaptation: To check for evolution of the populations after their introduction to laboratory culture, the measurements of size traits for the six populations examined in the present study after 2 yr in laboratory culture (see below) were compared with similar measurements made 9 mo after introduction to the laboratory (JAMES *et al.* 1995). Thorax length was also measured after 7 mo in the laboratory, and all possible combinations of time intervals were compared for this trait.

Phenotypic plasticity: These experiments were conducted ~2 yr after the flies were collected (<20 generations). We worked with a subset of the lines, two replicate sites from three latitudinal regions: (1) tropical Queensland (MO and IN, 16°53′S), (2) intermediate sites (GL, 25°33′S and BH,

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27°57′S) and (3) temperate Tasmania (FT, 41°11′S and RN, 42°53′S). These sites were chosen because they covered the full latitudinal range and were representative of the geographic differentiation for larval development time and body size (JAMES and PARTRIDGE 1995; JAMES *et al.* 1995). We reared flies from each line for two generations at 14, 18, 21, 25 and 29° (these temperatures span the range of viability and fertility of this species; DAVID *et al.* 1983). Eggs were collected from each population cage in yeasted bottles of food medium to produce uncrowded cultures and were transferred to the experimental temperature. Eclosing adults were transferred to "laying pots" containing a yeasted medium of grape juice and agar, allowed to acclimate to their new environment, given a pre-lay period to oviposit any retained eggs and then given fresh laying pots to lay the eggs that would hatch to give the experimental animals. Timing was adjusted to compensate for slower life-history processes at lower temperatures. Four 8-hr egg collection periods were used for the 14° experiment, two 4-hr collection periods were used in the 18° experiment, two 3.5-hr collection periods were used in the 21° and the 25° experiment and four 2-hr lays were used in the 29° experiment. Upon hatching, first instar larvae were transferred into yeasted vials of medium, 30 larvae per vial and 20 vials per site. Times of pupariation and eclosion were recorded. After eclosion, four males and four females from each replicate vial were randomly selected and one of their wings was used to measure wing area and cell area. From each of five vials per line and temperature, four males and four females were randomly selected and their thorax length was measured.

Measurements: Times of pupariation and eclosion were recorded, adjusting timing for the effects of experimental temperature. Vials were checked every 24 hr at 14°, every 12 hr at 18°, every 8 hr at 21 and 25° and every 6 hr at 29°. Larval development periods were estimated by subtracting the midpoint of egg lay from the average time of pupariation for each vial (as larvae were not sexed, sexes were combined). Pupal periods were calculated as the difference between average time of pupariation and average time of eclosion in each vial for each sex. Egg-to-eclosion times were determined by subtracting the midpoint of the egg lay from the average eclosion time in each vial for each sex.

Thorax length was measured to the nearest 0.02 mm with an eyepiece graticule under a dissection microscope at ×25 magnification, as the distance from the base of the most anterior humeral bristle to the posterior tip of the scutellum on the left side. Wings to be measured were fixed in propanol and mounted in Aquamount on a microscope slide. Wing area was measured using a *camera lucida* attached to a dissecting microscope at ×50 magnification, by tracing the outlines on a Quora graphics tablet connected to a Macintosh computer. Trichomes, in a standard 0.01 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. Average cell area was calculated by dividing 0.01 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 cell area. Although cell area varies throughout the wing, the changes in distinct regions in response to evolutionary and environmental temperature are concordant (DELCOUR and LINTS 1966; PARTRIDGE *et al.* 1994a), so using an index of total cell number based on one region is legitimate. Wing size traits were then linearized by taking their square root.

Statistical analyses: To compare latitudinal variation in the size characters (thorax length, wing area, cell area and cell number) of the field-caught and laboratory-reared flies, analysis of covariance for the effects of rearing environment (field collected or laboratory raised, discrete), latitude (continuous) and sex (discrete) as fixed main effects, were used. Since we were interested in differences in elevation only within the latitudes that we examined, centered latitudes were used in the analysis of covariance. This is the residual of the latitudes and thus tests if the differences occur within our transect rather than projecting the means to the intercept of latitude 0. To ensure proper comparison, laboratory data corresponding to those samples for which there were no field data were dropped from the analysis.

To test the possibility of evolution of size traits of the lines while in captivity analysis of covariance with rearing environment (time of measurement, discrete) and latitude (continuous) as fixed main effects were performed on thorax length, wing area, cell area and cell number (by sex).

In the phenotypic plasticity study variances among experimental temperatures were significantly heterogeneous for several traits (Levene's test: preadult development time in both sexes, $P < 0.01$; larval period and pupal period in males, $P < 0.001$; cell size in males, $P < 0.025$). The among-environment heteroscedasticity was eliminated for all traits by transformation following DUTILLEUL and POTVIN (1995, equation 6). This approach eliminates between-environment heteroscedasticity without changing the ranking of trait values across genotypes. Four of five cases showing significant heterogeneity of variances between environments produced different significance levels of results when analyzed on transformed and untransformed data. No such disparity occurred in homoscedastic characters. The residuals from the analyses of variance for all transformed traits were normally distributed (Shapiro-Wilk W test, $P > 0.05$).

To select a method of analysis of the plasticity data power analyses were performed on a variety of different statistical models [analyses of variance (ANOVAs), orthogonal polynomials and regressions] to determine which model produced the best fit. We set the significance level (α) to 0.05 and the power (ϕ) to 0.80, and used the square root of the mean square error from the data (σ) to define the variation within the data for each character. Given these parameters the raw effect size (δ) was found. To compare the models the ratio of σ:δ was calculated. This ratio measures the amount of variance the particular model is sensitive to (a number less than one means the model is sensitive to the variance in the data set, while a number greater than one means it is not).

Repeated-measures ANOVA, with temperature and latitude as crossed fixed effects and site as a random effect nested within latitude, had the highest power. For example, in detecting the temperature by latitude interaction in larval development time, the σ:δ ratios for linear and quadratic regressions were both 1.64, while they were 1.06 for the ANOVA model. Analysis that ordered the environments (*e.g.,* regressions and orthogonal polynomial) did not add information to the temperature, latitude by temperature or site within latitude results. In some cases ordering environments did detect clinal variation in a trait that the ANOVA analysis was not sensitive to. In these cases, the results of orthogonal polynomial analysis, with temperature to the forth polynomial, latitude to the quadratic polynomial and sites nested with latitude as a random effect are reported in the text.

To investigate the cellular basis of variation in wing area we followed a regression approach developed by ROBERTSON (1959) and STEVENSON *et al.* (1995). If cell area determines all the variation in wing area then the regression of log ($\sqrt{cell\ area}$) on log ($\sqrt{wing\ area}$) will have a slope of one; reciprocally, if the wing area variation is completely based on cell number, then that slope should be zero. Therefore, the slope of the regression of log ($\sqrt{cell\ area}$) on log ($\sqrt{wing\ area}$) indicates the relative contribution of cell area to changes in wing area. To

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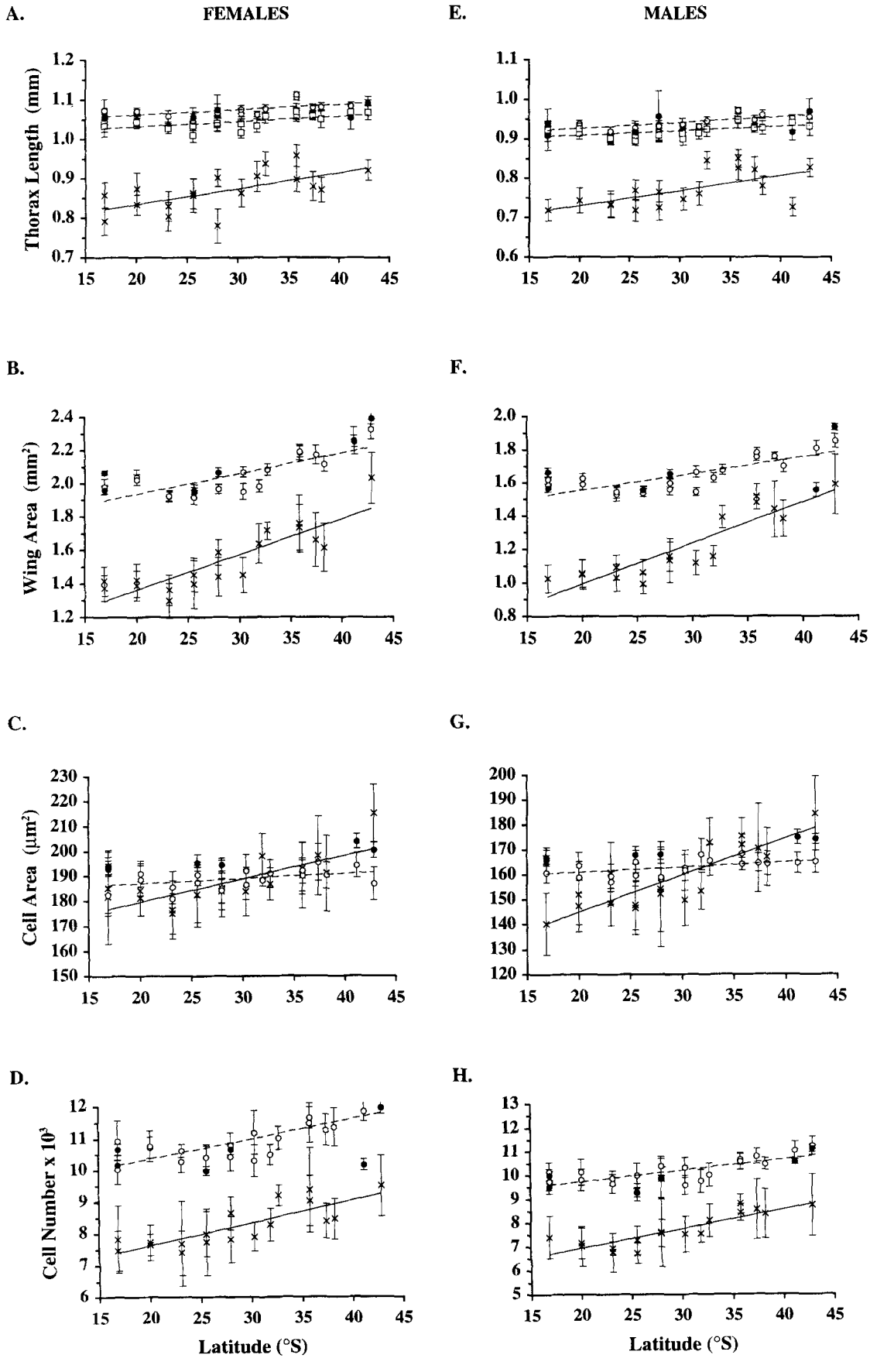

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TABLE 1

Analyses of covariance for body size characters of field collected and laboratory-reared (18°) flies

Character	Source of variation	MS	*F*	*P*
Thorax length	RE	7084.990	20990.110	***
	Latitude	13.800	40.890	***
	Sex	32.900	97.474	***
	RE × latitude	13.210	39.137	***
	RE × sex	26.990	79.970	***
	Latitude × sex	0.173	0.511	NS
	RE × latitude × sex	0.177	0.526	NS
√Wing area	RE	0.610	588.808	***
	Latitude	0.177	171.851	***
	Sex	0.382	369.050	***
	RE × latitude	0.029	27.654	***
	RE × sex	0.000	0.023	NS
	Latitude × sex	0.002	1.466	NS
	RE × latitude × sex	0.002	1.505	NS
√Cell area	RE	0.190	5.156	*
	Latitude	2.795	75.738	***
	Sex	19.439	526.670	***
	RE × latitude	1.322	35.825	***
	RE × sex	0.155	3.104	NS
	Latitude × sex	0.222	6.013	*
	RE × latitude × sex	0.083	2.246	NS
√Cell number	RE	3.212	824.226	***
	Latitude	0.439	112.505	***
	Sex	0.221	56.589	***
	RE × latitude	0.255	6.549	*
	RE × sex	0.001	0.167	NS
	Latitude × sex	0.004	0.002	NS
	RE × latitude × sex	0.005	1.138	NS

RE, rearing environment; MS, mean squares; NS, not significant. $P < 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

determine the cellular basis of plasticity with temperature and of variation among sites in wing area, we did analyses of covariance on log (√*cell area*) with main effects of site and temperature and log (√*wing area*) as the covariate, using the line means at each temperature. Sexes were analyzed separately.

All the analyses were done using the JMP package version 3.1 for the Macintosh (SAS 1994).

RESULTS

Geographical variation of body size in the field: Latitudinal variation in body size, cell area and cell number in field-caught flies was compared with measurements on the same lines with standard laboratory rearing at 18° by JAMES *et al.* (1995) and in the present study (Figure 1, Table 1). The flies collected in the field had smaller thoraxes and wings than the laboratory-reared flies, and their wings had fewer cells. All traits increased significantly with latitude. The slopes of the lines were steeper for the field-collected flies for all traits, although only marginally so for cell number. Cell area contributed significantly more to the wing area field cline, relative to the laboratory-reared flies, than did cell number (Figure 1, Table 1).

Laboratory adaptation: Analysis of covariance of the size traits for the six populations measured in the present study and at earlier stages in their laboratory culture revealed no temporal trends; neither the mean of the characters nor the interaction between rearing environment and latitude showed any evidence of change ($P > 0.2$ for the effects of time and the interaction of latitude with time for all characters).

Phenotypic plasticity of development time: Variation in development time components between experimental temperatures was high (explaining >99.9% of the variance in each trait) and far exceeded variation between sites (Table 2). Larvae that were raised in hotter environments pupariated sooner and had shorter pupal periods, resulting in faster preadult development time for both males and females. Flies from different latitudinal regions did not show significant differences in devel-

FIGURE 1.—Latitudinal clines of body size characters in nature and with standard laboratory rearing (linear regression lines on genetic clines and means with 95% confidence limits). All data points are shown, although only data present in all data sets being compared were used in analysis. (A) Thorax length, females. (B) Wing area, females. (C) Cell area, females. (D) Cell number, females. (E) Thorax length, males. (F) Wing area, males. (G) Cell area, males. (H) Cell number, males. ×, wild caught flies; □, laboratory reared after 7 mo captivity; ○, laboratory reared after 9 mo captivity; ●, laboratory reared after 2 yr captivity.

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TABLE 2

Repeated measures ANOVA on transformed development time data

Character	Temperature		Latitude		Site within latitude		Latitude × temperature	
	R^2	F	R^2	F	R^2	F	R^2	F
Larval development time	0.99967	57324.45***	0.00013	3.32	0.00006	4.41*	0.00010	2.77†
Pupal period (females)	0.99969	28417.740***	0.00006	1.88	0.00006	2.47	0.00006	0.84
Pupal period (males)	0.99977	55519.320***	0.00013	1.14	0.00007	5.41	0.00005	1.34
Preadult development time (females)	0.99975	72680.070***	0.00012	3.04	0.00006	6.27**	0.00001	0.50
Preadult development time (males)	0.99976	93248.730***	0.00012	2.36	0.00007	9.08**	0.00002	0.77

Repeated measures ANOVAs on transformed data, with temperature and latitude as crossed fixed effects and site as a random effect nested within latitude. The mean squares (MS) for latitude were tested against the MS for site within latitude (d.f. = 3). All other MS were tested against residual MS (population × temperature, d.f. = 12). † $P = 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

opment time. Flies from different sites within latitude showed significant variation for the larval period and the preadult periods, because flies from one of the high latitude sites (RN) took longer to develop than those from the replicate site (FT) for that latitudinal region (*a posteriori* contrast, $P < 0.01$; Figure 2). Latitudinal differentiation in phenotypic plasticity, as measured by the temperature by latitude interaction, was marginally significant for larval period but none of the other development time traits (Table 2). Larvae from the Queensland sites developed more slowly than larvae from other sites at the three intermediate temperatures (*a posteriori* analysis on partial data sets, $P < 0.05$). When raised at the two temperature extremes (14 and 29°), there were no significant differences in the larval period of populations from different latitudes. Patterns of total preadult periods were similar to those of the larval periods, but with more noise, as these traits combine the larval periods that showed the pattern and the pupal periods in which no significant pattern existed.

Phenotypic plasticity of body size: All body size characters showed a strong plastic response and decreased with temperature (Figure 3; Table 3). There were latitudinal trends in wing area and concordant effects on cell number in both sexes. Orthogonal polynomial analysis confirmed that the variation was clinal (linear latitude effect significant at the 0.05 level in each case). Thorax length and cell area showed no latitudinal variation (Table 3), although latitudinal variation did show up in cell area of males if analyzed by orthogonal polynomial analysis (linear latitude test, $P = 0.003$). Flies from the high latitude Tasmanian sites had larger wings with more cells than those from the other four sites (*a posteriori* contrast $P < 0.05$; Figure 3). No clinal variation in degree of plasticity was found in thorax length of males or in wing traits (Table 3), but there was a marginally significant effect for thorax length in females. Cell area was the principal determinant of the plasticity of wing area in response to temperature (Table 4). Variation between sites in wing area in the phenotypic plasticity experiment was mainly due to cell number and was consistent between rearing environments and experimental temperatures (Table 4).

DISCUSSION

Flies of both sexes captured in nature from all populations had smaller thoraxes and wings than those reared under standard conditions in the laboratory. This reduction in wing area was accounted for mostly by a reduction in cell number rather than in cell area. This finding suggests that the flies from nature were smaller because of poor nutrition or larval crowding, since these reduce mainly cell number (ROBERTSON 1959), unlike environmental temperature, which exerts its effects almost entirely by altering cell size (ALPATOV 1930; ROBERTSON 1959; DELCOUR and LINTS 1966; MASRY and ROBERTSON 1979; PARTRIDGE *et al.* 1994a). Higher temperatures in the field as an explanation of the reduced size of field-collected flies is implausible if the Tasmanian flies are considered. During the season they were collected, average daily temperature at their collection sites was 16.9°, lower than the temperature at which the laboratory measurements were made, yet the field-collected flies from these sites were smaller than their laboratory-reared counterparts. Another explanation for the larger size of the laboratory-reared flies could have been the evolution of larger body size under laboratory conditions. However, the marked similarity of the repeated measurements on these populations at 7 and 9 mo and 2 yr after collection argue against this possibility. In addition, only after intense divergent artificial selection on body size for 30 generations did size differences approach the observed magnitude of the difference between field-collected and laboratory-reared flies (PARTRIDGE and FOWLER 1993), and these collections had been in captivity for many fewer generations when first measured.

The latitudinal clines in thorax length and wing area were significantly steeper for flies collected in nature, and the increase in steepness in wing area was accounted for mostly by an increase in the steepness of the cline for cell area. These populations did not show

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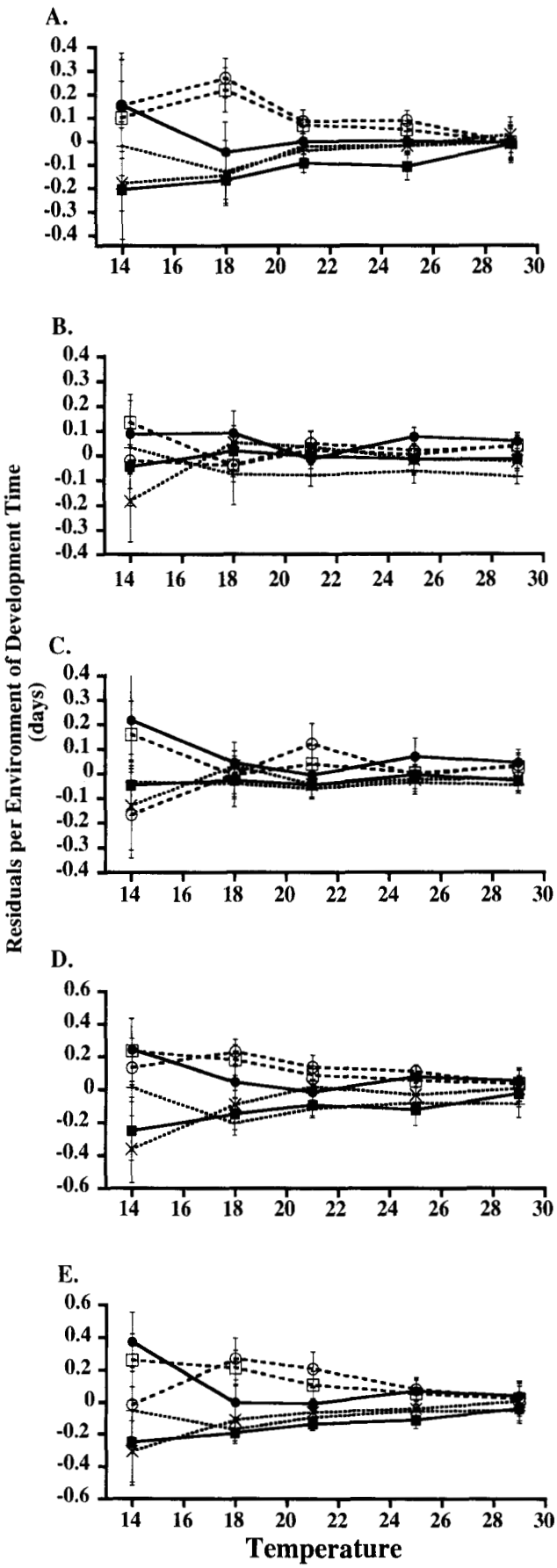

latitudinal variation in plasticity of size characters. The implication, therefore, is that latitudinal variation in environmental temperature in nature increased the steepness of the phenotypic cline. This finding supports the idea that the evolutionary and developmental responses of body size to temperature share a common function, since they show co-gradient variation in nature. The trend is also consistent with temperature as an important selective agent for body size in nature, since its environmental impact on size varied in the predicted way along the cline.

All the traits studied were plastic when subjected to different temperatures; flies raised in hot environments developed faster and eclosed as adults with smaller wings containing smaller and fewer cells. Plasticity of wing area in response to temperature was found to be mostly caused by changes in cell area, in accordance with previous studies (ALPATOV 1930; ROBERTSON 1959; DELCOUR and LINTS 1966; MASRY and ROBERTSON 1979; PARTRIDGE *et al.* 1994a). In contrast latitudinal variation in wing area was produced mainly by changes in cell number at all temperatures, supporting previous observations at a single temperature (JAMES *et al.* 1995).

No significant latitudinal variation was found for any of the development time traits. Marginally significant ($P = 0.05$) latitudinal variation in plasticity of larval development time in response to temperature was found. At intermediate experimental temperatures (18–25°) larval and preadult development times were longer in the populations from low latitudes, as has been previously reported (JAMES and PARTRIDGE 1995). However, development times did not differ between latitudinal populations at the experimental thermal extremes (14 and 29°), possibly reflecting that these conditions are stressful for all populations. Thus, latitudinal variation in plasticity seems unlikely to be of biological significance in nature. Although statistically significant, the effect was of very small magnitude in comparison with the direct impact of environmental temperature.

Wing area showed geographic variation in plasticity, but there was no clinal trend, concurring with COYNE and BEECHAM's (1987) results for North American *D. melanogaster*. There was a marginally significant increase in plasticity of female thorax length with latitude, but the effect was small. The lack of latitudinal trends in plasticity is surprising. If the developmental and evolutionary responses of body size to temperature are both adaptive, and for the same reasons, then one might expect to see higher levels of plasticity in environments with more variable thermal regimes, which would be found at higher latitudes. A flexible, plastic response would, for instance, be able more accurately to track

FIGURE 2.—Thermal reaction norms of residuals per environment of the preadult development time characters (means and 95% confidence limits). (A) Larval period. (B) Female pupal period. (C) Male pupal period. (D) Female preadult period. (E) Male preadult period. ○, IN; □, MO; ×, GL; +, BH; ■, FT; ●, RN.

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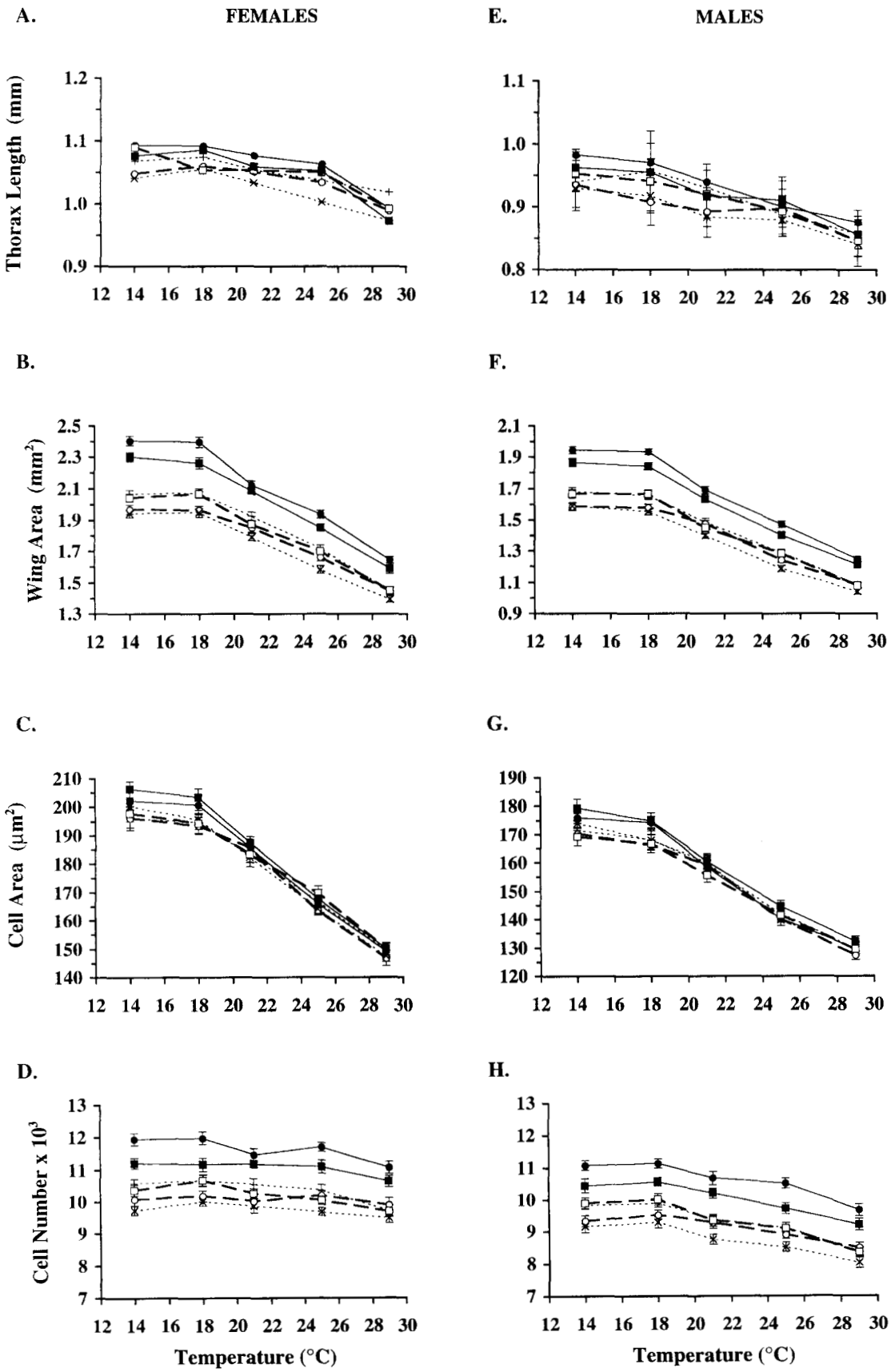

FIGURE 3.—Thermal reaction norms of body size characters (means and 95% confidence limits). (A) Thorax length, females. (B) Wing area, females. (C) Cell area, females. (D) Cell number, females. (E) Thorax length, males. (F) Wing area, males. (G) Cell area, males. (H) Cell number, males. ○, IN; □, MO; ×, GL; +, BH; ■, FT; ●, RN.

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TABLE 3

Repeated measures ANOVA on Transformed body size data

Character	Temperature		Latitude		Site within latitude		Latitude × temperature	
	R^2	F	R^2	F	R^2	F	R^2	F
Thorax length (females)	0.769	95.990***	0.063	1.09	0.086	14.33***	0.058	3.63*
Thorax length (males)	0.831	94.310***	0.089	3.21	0.041	6.26**	0.013	0.73
√Wing area (females)	0.780	1029.350***	0.194	12.58*	0.023	40.61***	<0.001	0.43
√Wing area (males)	0.826	992.370***	0.156	15.24*	0.015	24.54***	<0.001	0.27
√Cell area (females)	0.986	732.510***	0.006	4.23	0.002	2.07	0.002	0.66
√Cell area (males)	0.984	749.60***	0.008	5.12	0.002	2.38	0.001	0.78
√Cell number (females)	0.120	15.280**	0.725	8.53†	0.127	21.62***	0.004	0.27
√Cell number (males)	0.395	99.170***	0.501	8.26†	0.091	30.50***	0.001	0.13

Repeated measures ANOVAs on transformed data, with temperature and latitude as crossed fixed effects and site as a random effect nested within latitude. The mean squares (MS) for latitude were tested against the MS for site within latitude (d.f. = 3). All other MS were tested against Residual MS (population × temperature, d.f. = 12). † $P = 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4

Percentage of variation in wing area attributable to cell area

Source of variation	Females	Males
Phenotypic plasticity experiment		
Temperatures	83.4 ± 4.2	67.6 ± 3.0
Sites	13.4 ± 6.5	13.1 ± 6.9
Flies collected in the field		
Sites	35.3 ± 9.9	46.5 ± 9.0

Values are means and 95% confidence limits.

seasonal changes in temperature. The developmental mechanisms controlling the plastic response may have a range that is difficult to increase by selection. However, artificial selection for increased plasticity as a function of temperature has been successful (SCHEINER and LYMAN 1991), which argues against this explanation. The lack of increase in plasticity with latitude could be a consequence of gene flow from lower latitudes. The temperatures experienced during growth and breeding may also be more similar at different latitudes than the annual range of temperature variation would indicate, if the activity season is shorter at higher latitudes, as has been described for the Atlantic Silverside *Menidia menidia* (CONOVER and PRESENT 1990). Finally, if increased plasticity is costly, by reducing some other aspect of fitness, an evolutionary increase at higher latitudes might not be seen (VAN TIENDEREN 1991; GOMULKIEWICZ and KIRKPATRICK 1992).

Egg-to-adult development time has been divided into two distinct periods: the larval and the pupal period. Both components of development are plastic when subjected to different temperatures, but it is the larval period that shows genetic differences. This is the stage when the preadult fly is actively seeking food resources and converting these resources into larger body size. Differential development periods and resulting adult body size may be caused by selection on behavior or physiology at this stage. There is evidence that both may be in effect. RUIZ-DUBREUIL *et al.* (1996) found that feeding rate of young larvae positively correlated with fast development time, while faster feeding rate of older larvae resulted in a larger body size. Supporting physiological differences, evolution of flies at low temperature produced larvae that required less food to reach a given size (NEAT *et al.* 1995).

While development time and body size both respond to thermal selection, evolution of these two characters seem to be uncoupled. We have previously shown that while genetic clines in both these traits exist, there is no strong association between them among latitudinal regions (JAMES *et al.* 1995). The Queensland sites are exceptional with respect to development time, while the Tasmanian sites are exceptional with respect to the body size. Further evidence supporting the independence of these two traits comes from artificial selection on thorax length in *D. melanogaster*. Flies selected for large thorax length extended their development time (PARTRIDGE and FOWLER 1993), which is the opposite response to the one seen with thermal selection.

In the present study, latitudinal variation has been implicated as a source of environmental variation in body size along the cline and as a selective agent on both size and development time. Future work should examine the mechanisms at work, including those determining the level of phenotypic plasticity.

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