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Cellular basis of wing size variation in *Drosophila melanogaster*: a comparison of latitudinal clines on two continents

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We investigated the cellular basis of two extensive, continuous, latitudinal, genetic, body size clines of *Drosophila melanogaster* by measuring wing area and cell size in the wing blade of adult flies reared under standard, laboratory conditions. We report that the contribution of cell size to an Australian cline is much smaller than that to a South American cline. The data suggest that neither cell size nor cell number were the targets of selection, but rather wing area itself, or a trait closely related to it. We hypothesize that the differences between the continents were caused by differences in the initial pattern of genetic variation for the cell traits and/or by the direction of selection on the source populations of the clines. Despite large differences between continents in the cellular basis of the latitudinal variation, multiple regression analysis, using the individual variation within populations, showed that the relationship between cell size and cell number was changed with latitude in the same way in the two clines. The relative contribution of cell number to wing area variation increased with latitude, probably because of compensatory interactions with cell size as a consequence of the latitudinal increase in cell number. Our findings are discussed in relation to the cellular basis of evolutionary change in laboratory thermal selection lines and natural populations along latitudinal clines.

Keywords: cell size, developmental regulation, latitude, temperature, thermal evolution.

Introduction

Latitudinal body size clines have been described for several ectotherms of different taxa: size increases with increasing latitude (Partridge & French, 1996). The most convincing data come from studies on the cosmopolitan fruit fly *Drosophila melanogaster*, probably because common garden experiments are relatively straightforward to perform. Such experiments have revealed genetic size differentiation along latitudinal clines on all major continents: Middle East–Africa (Tantawy & Mallah, 1961), Japan (Watada *et al*., 1986), North America (Coyne & Beecham, 1987), Eastern Europe/Central Asia (Imasheva *et al*., 1994), Australia (James *et al*., 1995, 1997) and South America (van 't Land *et al*., 1999).

Several findings have implicated temperature as the selective agent causing these body size clines. First, analysis in both the Australian and South American clines showed that latitude is highly correlated with minimum and maximum monthly and annual average temperature ($r < -0.90$), but not with annual average humidity, rainfall or sun hours ($r > -0.66$; data from Gentilli, 1971; van 't Land, 1997). Similarly, the body size of laboratory-reared descendants was significantly correlated with temperature but not with the other climatic variables (James *et al*., 1995; van 't Land, 1997). A second line of evidence for the importance of temperature as a selective agent in latitudinal clines comes from the finding that, in two independent cases, laboratory population cages of *D. melanogaster* kept at different temperatures evolved towards genetically larger size at lower temperatures (Cavicchi *et al*., 1985, 1989; Partridge *et al*., 1994).

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The overall size of an organism can increase because of larger cells, more cells or both. Thermal evolution in the laboratory has resulted in size differences, measured as wing area, that are a consequence of changes in cell size (Cavicchi *et al*., 1985; Partridge *et al*., 1994). In the Australian cline of *D. melanogaster*, the increase in wing area with latitude was caused by an increase in cell number, whereas cell size contributed only a small amount of the variation (James *et al*., 1995, 1997). Similar data are not available on other clines, and with so few studies it is hard to draw firm conclusions from the comparison of the cellular basis of size variation for laboratory lines and geographical populations. However, if latitudinal and laboratory thermal evolution result from similar selection pressures and targets of selection, then the different cellular bases of the responses so far reported imply that cell size or cell number *per se* cannot be the target of selection, which is likely to be body size itself. To help clarify this issue, in this paper we determined the cellular basis of size variation along a South American latitudinal cline (van 't Land *et al*., 1999), and compared it with the cellular basis of the Australian cline (James *et al*., 1995, 1997).

In addition, we investigated the relationships between cell number and cell size both between populations and between individuals within populations for the two continents. Studies of both phenotypic and genetic correlations have indicated that cell size and cell number are negatively correlated (Robertson, 1959a,b; Cavicchi *et al*., 1985; de Moed *et al*., 1997; Guerra *et al*., 1997; McCabe *et al*., 1997). Furthermore, when one of these traits is subjected to artificial selection, a negative correlated response is observed in the other, providing evidence for developmental compensation between cell size and cell number in the determination of total body size (McCabe *et al*., 1997; Conlon & Raff, 1999). Therefore, cell size and cell number are, at least in part, jointly regulated. Consequently, body size could differ between populations as a result of a change in cell size, cell number or both, perhaps without any change in the joint regulation of cell size and cell number. Alternatively, an evolutionary increase in total size could occur because the level of compensation between cell number and cell size was decreased, allowing a greater joint increase in the two traits. In this study we examined the role of compensation between cell size and cell number both in the production of latitudinal variation in body size and of variation between individuals within populations at different latitudes. Understanding how the regulation of wing development changes as a result of evolution should help explain the variation in the observed cellular basis of body size occurring in latitudinal clines and laboratory populations.

Materials and methods

Fly populations

South America We used flies collected by van 't Land and van Putten of the University of Groningen (van 't Land, 1997; van 't Land *et al*., 1999) during February and March 1995, at one location in Ecuador, and nine locations in Chile. In total, almost 40 latitudinal degrees were covered, whereas the longitudinal range of these locations was less than 10°. The number of collected females per site ranged from 22 to 334, with no correlation between genetic variation in the established populations and initial numbers of flies caught. The populations arrived in the Galton Laboratory, London in January 1996, and were subsequently maintained as discrete generations in uncrowded half-pint bottles at 25°C (12L:12D) and two bottles per population. About 11 months after field sampling, the experiment was started.

Australia We used data from the study of James and co-workers (James *et al*., 1995). The populations were collected along the eastern coast of Australia during February 1993. A range of 26 latitudinal degrees was covered and samples were taken from 13 latitudinal sites, of which seven were replicated. The field samples were established in the laboratory in population cages at 16.5°C from 30 isofemale lines and were transferred to 18°C after one year. The wing data (see later) were taken from experiments carried out within nine months of field sampling.

Experiments

Rearing Females from the South American populations were allowed to lay eggs in pots with removable food lids for 2 h. From the lids, eggs were picked at a density of 50 per vial. Each vial contained 7 mL of Lewis medium and five vials per population were picked (total of 50 vials). Development took place at 25°C. For the Australian population the rearing was similar, but larvae were picked at a density of 30 per vial, for 15 vials per population (James *et al*., 1995). Development took place at 18°C.

For both continents, the egg/larval density conditions were uncrowded and, for each temperature, the amount of food per larva/egg allowed maximal size to be attained (Economos & Lints, 1984; Zwaan *et al*., 1991). Both clines were measured in field-collected specimens

and on several occasions in the laboratory, and the elevation and slope of the latitudinal clines remained unchanged (James *et al.*, 1995, 1997; van 't Land *et al.*, 1999). Cell size, cell number and wing area for different populations from the Australian cline showed no significant interaction with experimental temperature over the full physiological range of temperatures, including those in the present study (James *et al.*, 1997). This implied that the regression and correlation comparisons between the clines in this study (see below) were not biased by the different rearing temperatures.

Wing measurements For the South American populations, 10 females and 10 males per vial were measured for the wing characters. Occasionally, fewer flies per vial were measured but always more then seven per sex. The left wing was removed, fixed in 2-propanol and mounted in Aquamount on a microscopic slide with a cover glass. Twelve vials (of the 15) per site were used for the Australian flies. Two to four wings per sex per vial were measured (James *et al.*, 1995).

Wing areas (mm^2) were measured at 50× magnification using a microscope with a *camera lucida* attachment and graphics tablet. The tablet was connected to a computer to store the data for later analysis. The outline of the wings was traced starting at the alar–costal break.

Each cell on the wing blade (two cell layers thick) secretes one hair, or trichome. Counting the numbers of trichomes in a standard area on the wing allows estimation of cell size. Wings were viewed down a compound microscope at 10×40 magnification, and a 0.01-mm^2 sampling square area was used, placed in the posterior medial cell of the wing, chosen by eye at equal distances from the fourth longitudinal vein, the posterior cross vein and the fifth longitudinal vein (McCabe *et al.*, 1997) (in that paper, figure 1, area 1). Cell size was calculated by [0.01 mm^2/no. trichomes]. An estimation of cell number was obtained with [wing area/cell size]. Cell size is variable throughout the wing, but variation between individuals is concordant for different measurement regions on the wing (Partridge *et al.*, 1994; McCabe *et al.*, 1997).

Analysis and statistics

All analyses were carried out using JMP version 3.2.2, except for the multiple factorial Levene's homogeneity of variance tests, which were performed in MINITAB release 12.21.

Latitudinal trends in wing traits The latitudinal trends in wing area, cell size and cell number for the Australian populations have been published elsewhere (James *et al.*, 1995). In this paper we do the same for the South American cline, analysing wing area, cell size and cell number. The variances for wing area and cell number were homogeneous (Levene's test, with population and vial as factors). The same applies to cell size for males, but not for females (Levene's test, $P < 0.036$). However, no correlations between the standard deviation or coefficient of variation with latitude were found for cell size in females. Analyses of variance (ANOVA) were carried out with wing area, cell size or cell number as dependent variable, and population, sex and vial nested within population as factors. The residuals from these ANOVAS were normally distributed (Shapiro–Wilk W-test). In addition, we performed an analysis of covariance for wing area, cell size or cell number, with sex as the independent factor and latitude as the co-factor. In this analysis population means were used and the means were obtained by, for each population, first averaging per vial and then averaging over vials.

The cellular basis of wing area variation The relative contributions of cell size and cell number to variation in the size of a body part, among individuals, populations or species, can be determined by considering the covariation of log-transformed body part size and cell size (Robertson, 1959a; Stevenson *et al.*, 1995). Let W be log(wing area), C be log(cell size) and N be log(cell number). Because wing area is the product of cell number and cell size, we have:

$$W = C + N. \tag{1}$$

When we fit the following regression models:

$$C = a + b \cdot W \tag{2}$$

and

$$N = c + d \cdot W. \tag{3}$$

The regression slopes are given by the formulae:

$$b = \mathrm{Cov}(C, W)/\mathrm{Var}(W)$$

$$d = \mathrm{Cov}(N, W)/\mathrm{Var}(W).$$

From these formulae and using property (1) it can be shown that:

$$\mathrm{Cov}(C, W) + \mathrm{Cov}(N, W) = \mathrm{Var}(W). \tag{4}$$

Therefore,

$$b + d = 1. \tag{5}$$

From the above formulae, we can conclude that slope b estimates the proportion of the variance in wing area

explained by its covariation with cell size. In contrast, regression models analogous to (2) and (3), but in which wing area is treated as a dependent variable, do not possess property (5) and therefore cannot be interpreted in the same way (Stevenson *et al*., 1995).

Between populations In an analysis of the cellular basis of a latitudinal cline, the slope b describes the contribution of cell size to the latitudinal cline in wing area. A slope b of 1 indicates that all wing area differences can be accounted for by cell size. Positive slopes <1 occur when both cell number and size contribute to wing area variation and when $b = 0.5$, cell size and number change isometrically. A slope of 0 means that cell size variation is uncoupled from wing area variation. For this analysis we used the population means for both continents following the method given above. However, this analysis can potentially be confounded with the inter-population deviation from the clinal relationship. In order to test whether deviations from the cline were affecting the results, we first regressed log(wing area) and log(cell size) on latitude and stored the fitted and residual values. Subsequently, we carried out separate regression analyses for log(cell size) on log(wing area) using the fitted and the residual values.

Compensation between cellular components within populations To detect changes in the relationship between cell number and cell size across individuals with latitude, vial means will not suffice and data on individual flies must be used. The response variable, log(cell size), should satisfy the assumptions of ANOVA: homogeneity of variances and normality of errors. An analysis of variance was performed on log(cell size) with population and sex as fixed effects and vial nested within population. No significant effects of vial within populations were found in either continent ($P > 0.05$). The residuals of log(cell size) were normally distributed for both continents (sex, population and vial within population removed). In males, the variance was not heterogeneous among populations (Levene's test, with population and vial as factors). Significant deviations from equal variances were found for the South American females ($P < 0.034$), but not for Australian females. However, neither the standard deviation nor the coefficient of variation of log(cell size) were significantly correlated with latitude. Therefore, the use of the whole data set in the analysis of latitudinal trends in the cellular basis of size variation between individuals appears valid: the infringement of the assumptions underlying linear statistics should not result in false positives for latitudinal trends.

Within populations, the slope b indicates levels of compensation and, possibly, overcompensation. Slope values between 0.5 and 1 indicate that cell size is increasing more rapidly with wing area than cell number (cell number compensation). Slope values between 0 and 0.5 indicate that cell number is increasing more rapidly than cell size (cell size compensation). Cell number over-compensates for increases in cell size if the slope is greater than 1 (wing area increases more slowly than cell size because cell numbers are decreasing; $b > 1$). Conversely, cell size over-compensates for increases in cell number if the slope is negative (wing area increases, but cell size decreases; $b < 0$).

We also calculated Pearson's product–moment correlation coefficients between log(cell size) and log(cell number) for both sexes for the populations on both continents, in order to describe the sign and the degree of interdependence of the variables. Per continent, possible latitudinal trends in this interdependence were tested in a multiple regression analysis of z-transformed coefficients, with sex and latitude as factors. If the correlations for each population are large and negative, changes in the slope b with latitude must involve compensation.

In order to detect changes in the slope b, for both continents, a multiple regression model was constructed with log(cell size) as the dependent variable and sex, latitude and log(wing area) as predictors. The interactions between the predictor variables can then be used to infer changes in the slope b and the regulation of the cell traits. If a significant interaction between latitude and log(wing area) is found, changes in compensation have occurred, and then the sign of the parameter estimate tells us the direction of changes in compensation. When the sign is positive, the contribution of cell size to wing area variation is increasing with latitude, hence cell number compensates for increases in cell size. Alternatively, when the sign is negative, the contribution of cell size to wing area variation is decreasing with latitude, hence cell size compensates for cell number increases. The interaction terms in the multiple regression model should be tested against the variation between vials within each population. However, vials cannot be nested within the covariate latitude. We carried out the following analysis to circumvent this problem.

First, an analysis of covariance (ANCOVA) was carried out with log(cell size) as dependent, population, sex and vial within population as factors, and log(wing area) as covariate. A (quasi) minimum adequate model was found by a stepwise backward deletion procedure (Crawley, 1993). The highest-order interaction was removed from the full model if it did not explain a significant proportion of the residual variance ($P > 0.05$), and a reduced model was fitted. The next highest-order nonsignificant interaction with the highest P-value was then removed and a new reduced model was

fitted. Nonsignificant factors or interactions were maintained in the model when any higher-order interactions including the factor or interaction were significant. The factors and interactions remaining in the ANCOVA after this procedure were used to build the multiple regression model for log(cell size), replacing population with latitude.

Secondly, for this multiple regression model, the mean squares of the interaction terms were tested using the mean squares of the denominator from the corresponding ANCOVA, replacing latitude with population (with a concomitant change in the degrees of freedom).

Results

Latitudinal trends in wing traits

Significant latitudinal trends were observed for wing area, cell size and cell number on the South American continent for both sexes (Fig. 1).

Values for males were smaller for all traits, but the sexes had similar slopes (sex × latitude, $P > 0.05$; Table 1a). This paralleled the results from the Australian continent (James *et al.*, 1995). The small flies from the Guayaquil (2°13″S) population could be the main cause of the significant latitudinal clines. However, the conclusions were identical for wing area when the data were analysed without the Guayaquil population (Table 1b). No significant latitudinal clines were found for cell size and cell number when the Guayaquil population was omitted (Table 1b). This result can largely be explained by the fact that both variables explained about half of the variation in wing area (see below), hence their individual slopes with latitude were shallower than the slope of wing area with latitude.

Although the latitudinal clines were pronounced, individual populations sometimes deviated considerably from the regression line. This was most noticeable for the Iquique population (20°13″S) which had very large cells, but relatively few of them.

The two clines were very similar in slope for wing area. The main difference between the continents was found in the latitudinal cline for cell size. James and co-workers (James *et al.*, 1995) reported that cell number was the major contributor to wing area variation in Australia; a significant correlation between cell size and latitude was found only in males, and with a shallow slope compared to wing area and cell number. Here we report for the South American continent a strong cline for cell size in both sexes. In the next section we concentrate on describing the precise nature of the difference between the continents.

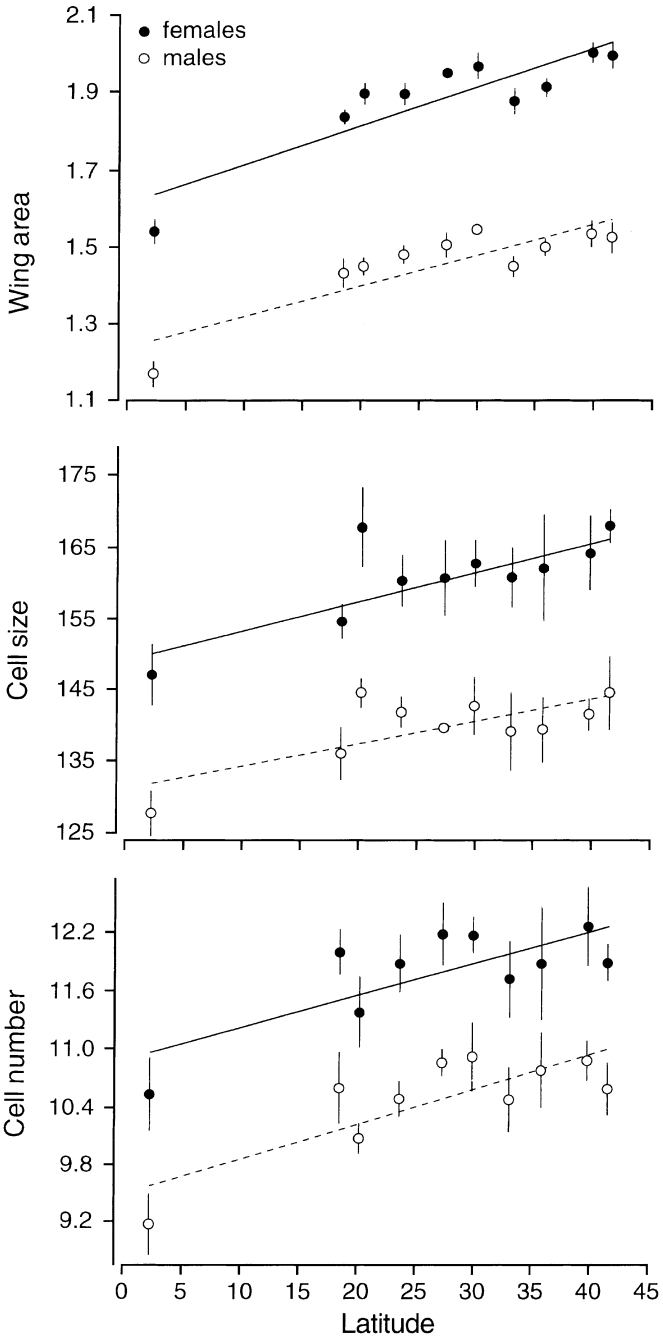

Fig. 1 The relationship between wing area (mm^2), cell size (μm^2) and cell number (thousand of trichomes), and latitude for South American female and male *Drosophila melanogaster*. The population means and 95% confidence intervals are based on vial means. All clines are significant (Table 1a). Regression lines, wing area (females, $y = 0.010x + 1.613$, R^2 adjusted 0.754, $P < 0.001$; males, $y = 0.008x + 1.240$, R^2 adjusted 0.708, $P < 0.01$); cell size (females, $y = 0.413x + 149.4$, R^2 adjusted 0.561, $P < 0.01$; males, $y = 0.308x + 131.2$, R^2 adjusted 0.477, $P < 0.025$); cell number (females, $y = 33.01x + 10882.7$, R^2 adjusted 0.521, $P < 0.025$; males, $y = 35.86x + 9489.9$, R^2 adjusted 0.615, $P < 0.01$).

Table 1 Analysis of covariance for wing area, cell size and cell number for the South American cline in *Drosophila melanogaster*

Source of variation	d.f.	(a) Wing area MS	(a) Wing area F	(a) Cell size MS	(a) Cell size F	(a) Cell number MS	(a) Cell number F	(b) Wing area MS	(b) Wing area F	(b) Cell size MS	(b) Cell size F	(b) Cell number MS	(b) Cell number F
Sex	1	0.1000	25.47****	2.40×10^{-10}	16.37***	13.80×10^{5}	11.09**	0.0417	31.49****	0.83×10^{-10}	7.21*	5.78×10^{5}	8.29*
Latitude	1	0.2012	51.26****	3.22×10^{-10}	22.01***	29.37×10^{5}	25.34****	0.0198	14.98**	0.31×10^{-10}	2.67	1.92×10^{5}	2.75
Sex × latitude	1	0.0026	0.65	0.07×10^{-10}	0.47	0.05×10^{5}	0.05	0.0008	0.64	0.06×10^{-10}	0.50	0.05×10^{5}	0.06
Error	16[a], 14[b]	0.0039		0.15×10^{-10}		1.16×10^{5}		0.0013		0.12×10^{-10}		0.70×10^{5}	
R^2 adjusted		0.937		0.901		0.835		0.975		0.913		0.865	
Slope		0.009		0.004×10^{-4}		34.399		0.004		0.002×10^{-4}		13.234	

Analyses were based on population means, with Sex as a fixed effect and Latitude as a covariate. Results are given for the data set including (a) and excluding (b) Guayaquil.
$^{*}P < 0.025$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

The cellular basis of wing area variation

Between populations Figure 2(a) shows the regressions of log(cell size) on log(wing area) for both sexes and continents. The slope of the regression line is significantly shallower for the Australian populations compared to the South American continent (females $t_{28} = 8.416$, $P < 0.0001$; males $t_{28} = 7.778$, $P < 0.0001$). The slopes estimate the contribution of cell size to wing area variation (see Materials and methods). Thus, cell size explained about half of the clinal variation in wing area for the South American populations and about a quarter for the Australian populations, and there was no indication of a difference in these trends between the sexes (Table 2a). For the South American cline, omitting Guayaquil from the analysis did not change the conclusions (data not shown).

The slope estimates were not different for the fitted data for both continents and sexes (Fig. 2a; Table 2b); the estimate of the contribution of cell size to wing area variation was very similar to that found in the first analysis (Table 2a). This result shows that the deviations from the latitudinal trends for wing area and cell size did not confound the examination of the cellular basis of the clines on the two continents. In addition, it shows that although population means may deviate from the cline, this did not affect the underlying cellular relationships. The slope estimate based on residuals was significant only for the South American cline, with the value being similar to the other two estimates (Fig. 2b; Table 2b).

Compensation between cellular components within populations For South America (Table 3), a significant interaction was found between latitude and log(wing area). No significant interaction was found between sex, latitude and/or log(wing area). This indicates that the effect of latitude on the relationship between log(cell size) and log(wing area) was similar for females and males: the contribution of log(wing area) to the variation in log (cell size) decreased significantly with latitude (Table 3). In the Australian populations (Table 3), all four possible interactions were significant. As a result, we analysed the sexes separately. It appeared that the significant interactions between factors and sex were caused by females, but not males, showing a significant interaction term of latitude × log(wing area) (analysis not shown). Again, the contribution of log(wing area) to variation in log (cell size) decreased significantly with latitude (Table 3). This indicates that with increasing latitude the contribution of cell size to wing area variation across individuals decreased and, conversely, the contribution of cell number increased.

Correlations between log(cell size) and log(cell number) were negative and highly significant for all

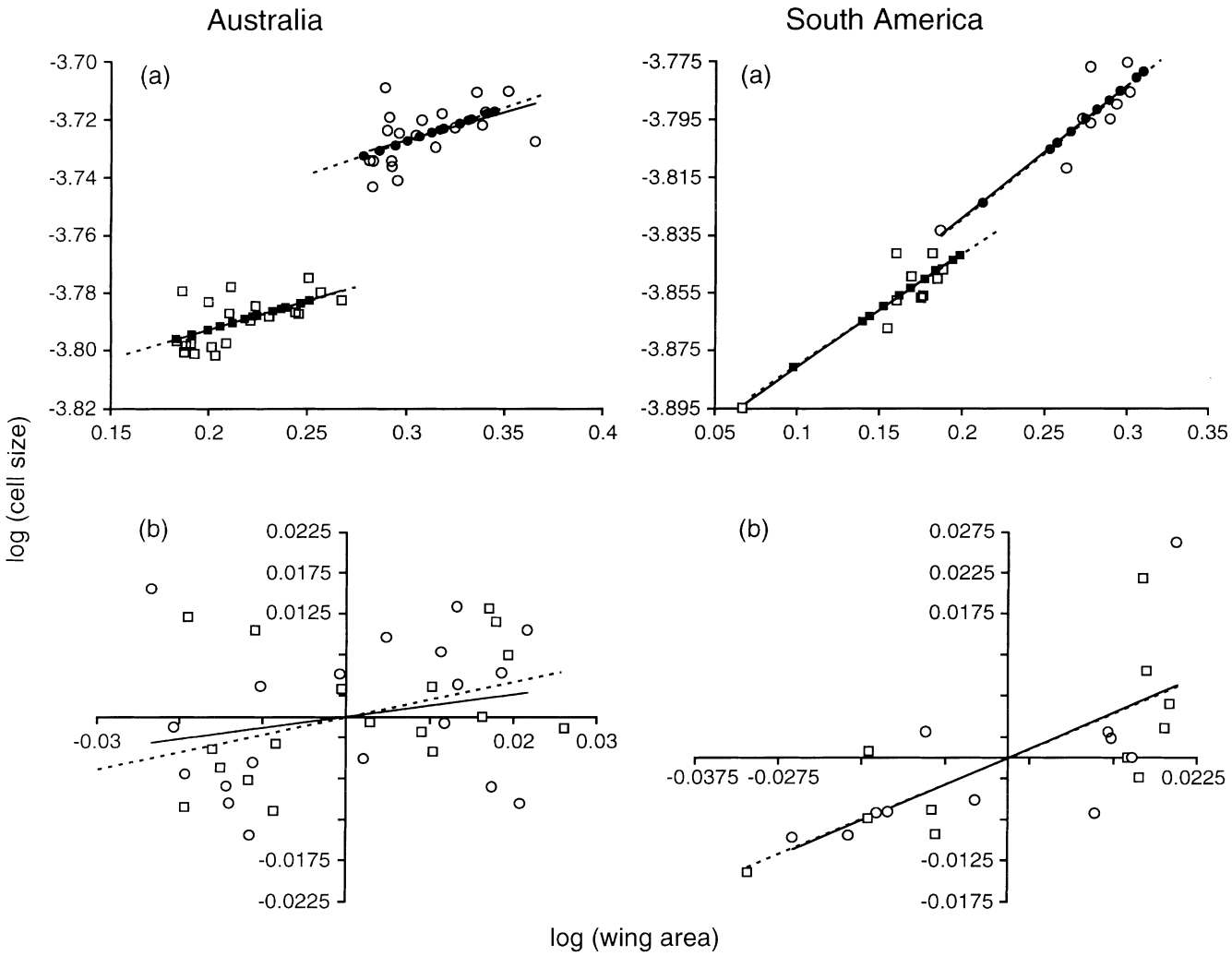

Fig. 2 (a) Regression between log(cell size) and log(wing area) for female and male *Drosophila melanogaster* from Australia and South America. Circles are females and squares are males. Open symbols are the population means (solid line); closed symbols are the fitted values of the variables with latitude (see text for details; broken line). (b) Regression between log(cell size) and log(wing area) for the residual values of the variables with latitude (see text for details) for females and males of Australia and South America. Circles are females (solid line) and squares are males (broken line). The slope of the regression line is an estimate of the contribution of cell size to wing area variation (Table 2a,b). Note: the size differences in the traits for the two continents were caused by the different rearing temperatures (see Materials and methods), but, for (a), the *y*-axis has the same scale-range.

populations and both sexes (mean (SE): Australian females, −0.745 (0.022); Australian males, −0.719 (0.022); South American females, −0.831 (0.012); South American males, −0.749 (0.025)). No latitudinal trends or interactions were found for the z-transformed correlation coefficients (data not shown), indicating that cell size and cell number remained tightly interdependent despite large changes in the absolute values of these traits. This, together with the functional relationship between cell size and wing area in the regression analysis, indicates changes in the compensation between cell size and cell number.

Discussion

Cellular basis of wing area: differences between the continents

Our results show that the cellular basis of latitudinal variation for body size is different for the continents of Australia and South America, with cell size playing a significantly greater role in the production of the South American cline. This would strongly suggest that wing area, not its cellular components, is targeted by natural

Table 2 Slopes *b* and standard error (SE) of the regression lines of log(cell size) on log(wing area) in *Drosophila melanogaster* for (a) the population means, and (b) the fitted and residual values from the regressions with latitude (see text for details)

				(b) Australia		(b) South America	
		(a) Australia	(a) South America	Fitted	Residual	Fitted	Residual
Females	Slope *b*	0.192**	0.459****	0.229	0.127	0.469	0.425*
	SE	0.080	0.085	—	0.132	—	0.176
	R^2	0.198	0.759	—	−0.004	—	0.350
Males	Slope *b*	0.199***	0.397****	0.196	0.206	0.388	0.423**
	SE	0.063	0.072	—	0.102	—	0.137
	R^2	0.326	0.767	—	0.140	—	0.485

The value of the slope represents the contribution of cell size to the latitudinal cline for wing area. The *P*-values test the null hypothesis that $b = 0$. The R^2 adjusted is given. Note that for the fitted values confidence intervals and R^2 are not informative because the model is saturated.
*$P < 0.05$; **$P < 0.025$; ***$P < 0.01$; ****$P < 0.001$.

Table 3 Minimum adequate model interaction terms of log(cell size), with sex, latitude and log(wing area) as predictor variables, for *Drosophila melanogaster*

	Australia			South America		
Source of variation	d.f.	Adjusted MS	*F*	d.f.	Adjusted MS	*F*
Latitude × sex	1	0.00931	13.69[A]***	1	0.00651	7.85[E]*
Latitude × log(wing area)	1	0.00511	7.51[B]*	1	0.00648	11.57[F]**
Sex × log(wing area)	1	0.00526	7.73[C]*	—	—	—
Latitude × sex × log(wing area)	1	0.00670	9.71[D]**	—	—	—
	Parameter estimates			Parameter estimates		
Latitude × sex	0.00275			0.00041		
Latitude × log(wing area)	−0.00738			−0.00570		
Sex × log(wing area)	0.23941			—		
Latitude × sex × log(wing area)	−0.00846			—		
R^2 adjusted	0.546			0.616		

*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.
Denominators from ANCOVA: [A]0.2987(sex × vial within population) + 0.7013 residual, MS = 0.00068, d.f. = 1009.9; [B]0.2991(log(wing area) × vial within population) + 0.7009 residual, MS = 0.00068, d.f. = 1018.4; [C]0.0152(sex × log(wing area) × vial within population) + 0.9848 residual, MS = 0.00068, d.f. = 873.25; [D]0.2991(sex × log(wing area) × vial within population) + 0.7009 residual, MS = 0.00069, d.f. = 1006.4; [E]0.84(sex × vial within population) + 0.16 residual, MS = 0.00083, d.f. = 52.364; [F]0.8667(log(wing area) × vial within population) + 0.1333 residual, MS = 0.00056, d.f. = 56.232.

selection. For *D. melanogaster*, or flying organisms in general, the wing aspect ratio (length/width) could be selected through its effects on flight performance at different temperatures; high values of wing aspect ratio might provide a fitness benefit at low temperature because it increases lift and compensates for the effects of ambient temperature on flight performance (Stalker, 1980; Azevedo *et al*., 1998). However, when reared under common garden conditions, wing aspect ratio did not vary with latitude in the Australian populations (Azevedo *et al*., 1998) and decreased with latitude in the South American populations (van 't Land *et al*., 1999). Therefore, larger body size (of which wing area is an indicator), or a trait closely associated to it, is the likely target of selection at higher latitudes. In accordance with this conjecture, thorax length of flies increased with increasing latitude for the Australian cline (James *et al*., 1995), and total body weight increased with increasing latitude for the South American cline (S. Robinson, B. Zwaan & L. Partridge, unpubl. results).

At least two factors may underlie the differences between the continents and/or constrain the cellular basis of wing area variation.

Genetic variation The availability of genetic variation for cell size and cell number may have been different for the founders of the two continental populations. Both Australia and South America were presumably colonized only a few centuries ago, and analysis of genetic variation for inversions, allozyme frequencies,

mitochondrial DNA variants and morphological and life history characters showed that significant founder effects have occurred on all continents (David & Capy, 1988). In addition, epistasis may have caused divergence in the nature and amount of genetic variation following colonization and founder events (Goodnight, 1988; Cheverud & Routman, 1995, 1996). Specific nonadditive and epistatic effects have been documented for the differences in wing area between cline-end populations (Gilchrist & Partridge, 1999) and on chromosome 3 for cell size (Robertson, 1959b; Zwaan & Partridge, unpubl.). It is therefore conceivable that the specific genetic constitution of the founders is still reflected in the cellular basis of the body size clines.

Developmental biology and direction of selection
Independent long-term (>25 generations) artificial selection experiments in our laboratory on thorax length and wing area using different wild-type strains, showed that increased body size was accompanied by an increase in cell number relative to controls, whereas decreased body size was the result of smaller cells in the wing blade (Partridge *et al*., 1999). The reasons for this asymmetry are not clear, but the different cellular basis of the body size clines might be caused by the direction of selection along the cline. The preponderant role of cell number in producing clinal variation in both continents could indicate that colonization has been mainly southwards in both cases, with evolution in general towards larger size as a consequence of more cells. Colonization and gene flow in the contrary direction could perhaps produce the impetus for the evolution of reduced size, realized mainly by reduced cell size. The Australian continent has been colonized from the north (David & Capy, 1988), in accordance with this hypothesis but, unfortunately, virtually nothing is known about the colonizing history of South America (David & Capy, 1988). Furthermore, as mentioned, laboratory thermal selection resulted in altered body size by altered cell size (Cavicchi *et al*., 1985; Partridge *et al*., 1994). This result may indicate that the populations used were originally adapted to the lower range of experimental temperatures used and, as a consequence, that evolution in the laboratory is predominantly towards a reduction in body size, which is therefore mediated mainly by a reduction in cell size. It would be helpful to have more data from lines derived by laboratory thermal selection to test these ideas. Laboratory evolution lines should be set up from populations with different thermal histories and different cellular bases for wing area variation. If laboratory body size evolution is consistently produced by changes in cell size, then the genetic and developmental reasons should be investigated.

Cellular basis of wing area: latitudinal effects on cellular relations in the wing

There is convincing evidence from *Drosophila* studies for a negative genetic relation between cell number and cell size (Robertson, 1959a, b; Cavicchi *et al*., 1985; Guerra *et al*., 1997; McCabe *et al*., 1997). In these studies both genetic and phenotypic manipulations of cell number resulted in compensatory effects of cell size, and vice versa. In this report, correlation analysis has shown a tight negative interdependence between cell size and cell number. In addition, in the functional analysis for both continents, a small but significant interaction was found between latitude and log(wing area): the contribution of cell number to the wing area variation between individuals increased with latitude, indicating that cell size compensated for latitudinal increases in cell number through wing area. Remarkably, the parameter estimate was of similar magnitude for the two continents. This finding supports the idea that wing area is the target of selection, and in the direction of larger wings with increasing latitude. The data provide no support for the idea that there are different selection targets along the two clines. In addition, the latitudinal effects on the relationship between cellular traits could in part explain the dominant role of cell number in the production of clinal variation in size. If cell number is more heritable than cell size when an evolutionary increase in total size is selected for (see above), then it will show the initial response, and compensation will inhibit the response in cell size. However, this compensation effect is small, and therefore the latitudinal increase of wing area indicates that the genes involved in producing the cline are mainly upstream of the regulation of cell size and cell number. The described pattern of compensation plays no role in producing the latitudinal body size cline and in fact opposes it.

In conclusion, we hypothesize that the differences between the continents were likely to have been caused by differences in genetic variation for the cell traits and their interactions and/or the direction of selection along the source populations of the clines. Despite these large differences, compensation between cell size and cell number has resulted in the relationship between these two cellular traits being changed with latitude in the same direction and by the same magnitude in the two clines.

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