Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis

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Summary

Eight isofemale lines of *Drosophila melanogaster* were raised at four temperatures and at four yeast concentrations in their food. Temperature and food show a significant interaction in determining wing length and thorax length, affecting mean size per line and genetic variation between lines. The combination of low temperature and poor food conditions leads to a sharp increase in the genetic variation over lines of both body size characters. The increase in genetic variation in wing length under less favourable conditions is due to an increase in genetic variation of both cell size and cell number. Changes in wing area in response to both temperature and food level follow a common cell size/cell number trajectory. Changes in wing size are obtained by line-specific changes in the cellular composition of the wing, rather than by changes specific for the environmental factor.

1. Introduction

Genetic variation in body size in *Drosophila* has received considerable attention (e.g. Imai, 1933; Robertson, 1955; Partridge & Fowler, 1993; see Roff & Mousseau, 1987). It was soon recognized that the heritability of body size characters depends on the environmental conditions during larval growth. Artificial selection experiments revealed higher heritabilities of wing length and thorax length at lower temperatures (Imai, 1933; Druger, 1962; Tantawy *et al.*, 1964, but see Tantawy, 1961) and lower food qualities (Robertson, 1960). Hoffmann & Parsons (1991) concluded that environmental stress generally leads to higher heritabilities, mainly due to higher levels of expressed genetic variation.

The effect of environmental factors on the expression of genetic variation in populations of *Drosophila* has been studied by comparing the reaction norms of isofemale lines. Genetic variation in plasticity, i.e. in the slope of the reaction norms, can lead to changes in genetic variation with the environment, showing a reduction of expressed genetic variation in environments in which reaction norms meet. Most studies on the variation in reaction norms of body size in *Drosophila* populations have focused on the effects of temperature (Thomas & Barker, 1993; David *et al.*, 1994; Barker & Krebs, 1995; Noach *et al.*, 1996; Morin *et al.*, 1996). Genetic variation depends on temperature in some populations, but not in all (Noach *et al.*, 1996). All these studies were performed under optimal food conditions. A reduction of yeast concentration in the food leads to an increase in both genetic and environmental variation in body weight (Gebhardt & Stearns, 1988, 1993). The only study on the effect of both temperature and food level on the genetic variation of body size was by Gebhardt & Stearns (1993), who reported no clear interaction between temperature and yeast concentration in the food on the genetic variation in body weight. It is not clear whether the two environmental factors interact in determining wing size.

Wing size is determined by two components: the size of the cells and their number. The cellular basis of wing size variation has been reported to differ with the source of the variation. Robertson (1959) and James *et al.* (1995) found that genetic differences in wing size under optimal conditions were mainly due to differences in cell number. A number of studies reported that changes in wing size in response to temperature led to changes in cell size and, to a lesser extent, in cell number (Alpatov, 1930; Robertson, 1959; Masry & Robertson, 1979; Partridge *et al.*, 1994). Alpatov (1930) and Robertson (1959) observed that increased larval densities or reduced food conditions led to a

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stronger reduction in cell number compared with cell size. The observed difference in cellular response to temperature and food availability suggests that cell size and cell number can be affected independently by environmental conditions, instead of the conditions affecting wing size as a whole. However, no studies are available on the interaction of temperature and food availability in the cellular composition of the wings in *Drosophila*.

In the present experiment we studied the variation between isofemale lines in reaction norms of wing length and thorax length in response to temperature and food level. We investigated the interaction of the responses of body size to both environmental factors and its effect on the expressed genetic variation in different environments, testing the independence of the environmental effects. Further, we studied whether the responses of wing size to temperature and food availability are obtained by separate changes of the two components in the cellular composition of the wing. We tested the hypothesis that temperature and food have predominantly separate effects on wing composition. The alternative would be an overall regulation of the cellular composition of the wing.

2. Materials and methods

(i) *Lines*

Fertilized females were collected in August 1991 from a compost container near Utrecht, the Netherlands, and used to start isofemale lines. Eight lines were used in the experiment. Lines were kept on standard corn meal medium at 17·5 °C, using single bottles containing 100–200 flies for 12 generations before the start of the experiment.

(ii) *Experimental conditions*

Lines were transferred to a Mittler–Bennet diet (56 g dead yeast, 54 g sucrose, 19 g agar and 1 mg nipagine in 1 litre tap water) at 25 °C for three generations before the experiment to reduce acclimation effects to the experimental media.

Larvae were grown at four temperatures (15 °C, 20 °C, 25 °C and 27·5 °C) on four food levels (one optimal food level and three levels with reduced yeast concentrations). These food-limited conditions led to a marked reduction in growth rate (De Moed *et al.*, unpublished data), but no marked reduction in viability. Experimental medium consisted of either 8 g, 12 g or 20 g of dead yeast with 20 g sucrose, 19 g agar and 1 mg nipagine in 1 litre tap water. Larvae were grown under axenic conditions to get repeatable and well-defined media. The fourth, optimal food level was created by growing larvae on standard corn meal medium with live yeast.

Eggs were sterilized in a 3 % hypochlorite solution for 15 min (Sang, 1956) and transferred to 30 ml vials containing 5 ml of medium with 30 eggs per vial. For each of the 16 temperature and food conditions two or three replicate vials were used. From each replicate vial five females were randomly taken for analysis.

(iii) *Morphology*

The right wing from each female was mounted on a microscope slide, embedded in Euparal. Wing length was determined on the mounted wing, taking the distance between the anterior cross vein and the distal end of the third vein. Thorax length was taken from the distal tip of the scutellum to the anterior margin of the scutum, using a dorsal view. Both wing length and thorax length were measured using a Reflex 3-D measurement microscope, accurate to ± 5 μm (Reflex Measurement Ltd, Butleigh, Somerset, UK).

(iv) *Cell size and cell number estimates*

Cell size was measured by counting the number of trichomes in a fixed area on the dorsal wing surface. Each bristle represents exactly one epidermal cell (Dobzhansky, 1928). The number of bristles were counted in a 0·0142 mm² area between the third and fourth vein, opposite the posterior cross vein, using an IBAS image analyser. Cell density is relatively homogeneous in this area (Schatz, 1951) and therefore rather insensitive to small changes in position of the counted area.

Wing area was estimated by the squared wing length, which is highly correlated with the surface area of the wing blade (data not shown). Cell number was estimated by dividing total wing area by the average cell size in the census area, or $\ln(\text{cell number}) = 2 \times \ln(\text{wing length}) - \ln(\text{cell size})$.

(v) *Analyses*

Variance components were estimated for each environment using nested one-way ANOVA, providing confidence limits for the between-line variance V_L (Sokal & Rohlf, 1981). V_L provides a measure of the genetic variation between lines (Hoffmann & Parsons, 1988). Estimates of genetic variation (V_G) based on between-line variation of isofemale lines depend on the variance in allele frequency, expressed as an inbreeding coefficient (Falconer, 1981, p. 241); the expected between-line variance is $2FV_G$, the expected within-line variance $(1-F)V_G$. Newly established isofemale lines are expected to show an F of 0·25. At a population size of 50, genetic drift during 12 generations is expected to raise F to 0·33 (Hoffmann & Parsons, 1988). However, due to the variation in fecundity between females and in male mating success, the effective population size may be smaller. Further, the introduction of the lines to the laboratory might lead to selection for performance under laboratory

conditions and to an erosion of genetic variation within isofemale lines. Heterozygosity within lines might therefore be limited and within-line variation V_W can therefore be expected to represent mainly environmental variation (V_E). The lines were fully viable and showed no signs of inbreeding depression.

V_W was estimated by summing the between-vial variance and the within-vial variance. Due to the use of axenic media between-vial variance in wing length and thorax length is generally small. Not all data on wing and thorax length could be linked per individual, and estimates of V_W of the wing to thorax ratio were based on between-vial mean squares.

Analysis of variance used a mixed model, taking temperature and food level as fixed effects and lines as random effects. Environmental factors were tested against their appropriate Line × Environment interaction term (Sokal & Rohlf, 1981). All statistical analyses were performed using SPSS/PC + v5.01 (SPSS, 1992).

3. Results

(i) *Reaction norms of body size characters*

Wing length and thorax length showed a marked difference in average response over lines to temperature and food conditions during development (Fig. 1 *a*, *b*). Wing length showed a strong linear reduction with temperature under optimal food conditions. Thorax length was hardly affected by temperature under optimal food conditions. Under food-limited conditions, the response of wing length levelled off at low temperature, while thorax length showed a maximum at intermediate temperatures. Both characters were highly sensitive to a reduction of food level. ANOVA showed a significant interaction between the effects of temperature and food level for both body size characters (Table 1 *a*, *b*). A reduction of food level induced a stronger response in wing length and thorax length at the extremes of the temperature range than at intermediate temperatures.

Significant genetic variation could be shown in the reaction norms of the both body size characters (Table 1 *a*, *b*). The significant Temperature × Line and Food × Line interactions for wing length and thorax length showed that the reaction norms of the isofemale lines are not parallel, but differed in shape.

Wing length and thorax length were highly correlated within temperatures and food levels over lines (Fig. 2). The relation between wing length and thorax length is generally analysed using their ratio (W/T ratio; e.g. David *et al.*, 1994; Barker & Krebs, 1995). The use of this ratio to correct for variation in general size implicitly assumes an isometric relation of wing length and thorax length between replicates. Over replicate vials, we observed a slope of the reduced major axis between wing length and thorax length not significantly different from 1 ($b_{rma} = 0.886$, 95 %
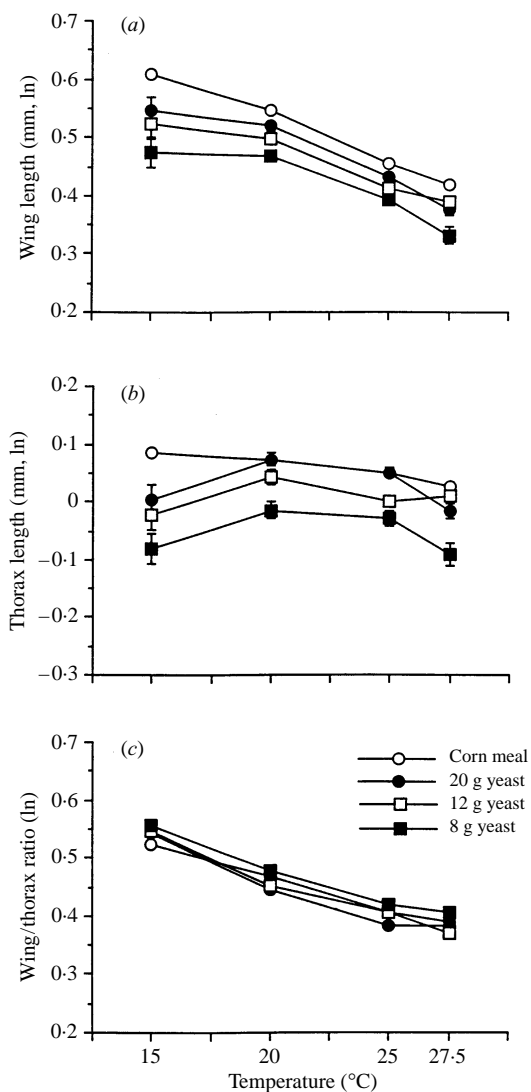

Fig. 1. Mean wing length (*a*), thorax length (*b*) and wing/thorax ratio (*c*) ($\pm$ SEM) in relation to temperature and food level. The standard errors may be too small to appear.

confidence interval: 0·769–1·025), justifying the use of a W/T ratio.

The reduction in wing length with food level was accompanied by a largely parallel reduction in thorax length (Fig. 2). The difference between wing length and thorax length in their response to temperature led to a reduction of their ratio at increasing temperatures (Table 1 *c*, Fig. 1 *c*). Food conditions had a smaller, but significant, effect on the W/T ratio. The lowest food level showed an increase in W/T ratio (Fig. 1 *c*).

Lines differed significantly in their W/T ratio (Table 1 *c*). However, no significant genotype × environment interaction could be shown for either temperature or food level.

(ii) *Genetic variation in body size*

The between-line variation V_L, which represents genetic variation (see Materials and Methods), showed

Table 1. *Analysis of variance of vial means of* (*a*) *wing length,* (*b*) *thorax length and* (*c*) *wing/thorax ratio*

Source	d.f.	(*a*) Wing length		(*b*) thorax length		(*c*) Wing/thorax ratio	
		MS	*F*	MS	*F*	MS	*F*
Environment							
Temperature (T)	3	45·21	88·77***	5·19	62·43***	39·00	957·50***
Food (F)	3	12·09	88·72***	17·52	210·90***	0·99	24·22***
T × F	9	0·49	4·29**	1·09	13·18***	0·22	5·51***
Genotype							
Line (L)	7	2·41	34·61***	2·65	31·93***	0·27	6·52***
L × T	21	0·51	7·30***	0·55	6·56***	0·05	1·30
L × F	21	0·14	1·95**	0·31	3·74***	0·05	1·30
L × T × F	63	0·12	1·65**	0·19	2·29***	0·07	1·68**
Error	181	0·07		0·08		0·04	
Total	309	0·74		0·48		0·45	

Each character was log-transformed.
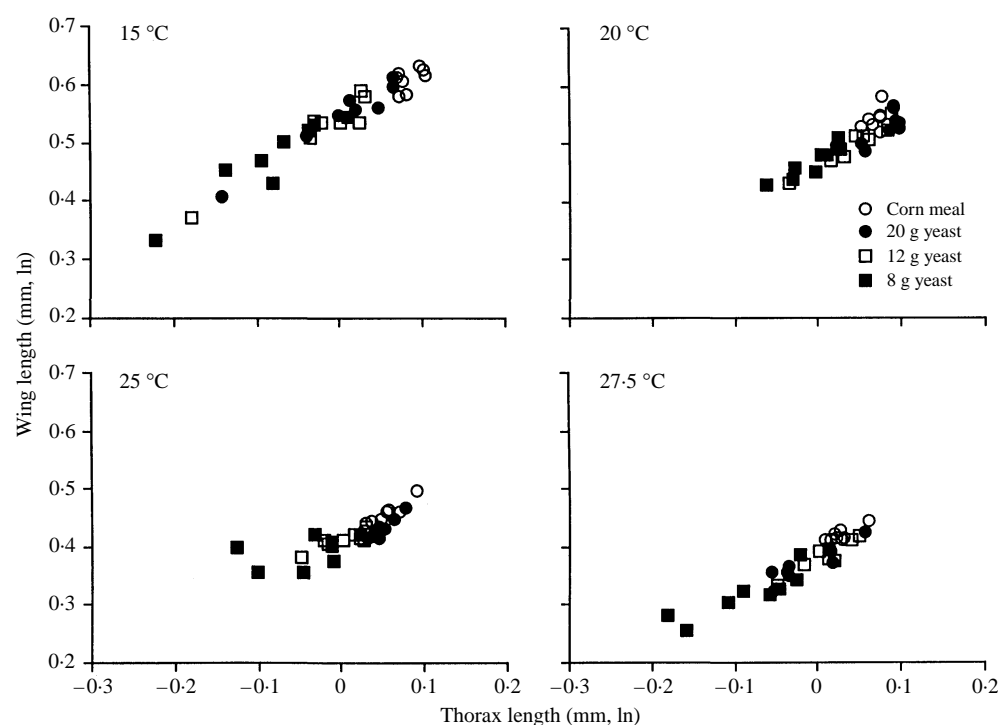* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 2. Mean thorax length and wing length per line at each food and temperature condition.

marked differences between environments, for both wing length and thorax length (Fig. 3). Under optimal food conditions, V_L was consistently low for each temperature. However, under food-limited conditions V_L of wing length sharply increased at lower temperatures. V_L of thorax length increased at both temperature extremes. The expression of high levels of genetic variation was achieved only by the combination of poor food conditions and extreme temperatures.

Within-line variation V_W partly consists of genetic variation left within lines, as well as environmental variation. However, the distinct patterns in V_L

(reflecting V_G) could not be traced in V_W (Fig. 3). V_W was hardly affected by temperature and a reduction in food led to an increase in V_W at each temperature. These results indicate that relatively little genetic variation remained within lines (see also Materials and Methods).

The W/T ratio showed little difference in V_L over environments. A small increase in V_L was observed at the two highest temperatures only at the lowest food level. In general, V_L of the W/T ratio was consistently low, but present in each environment (Table 1*c*). V_W increased at both extreme temperatures under food-limited conditions. It appears that the highly regulated
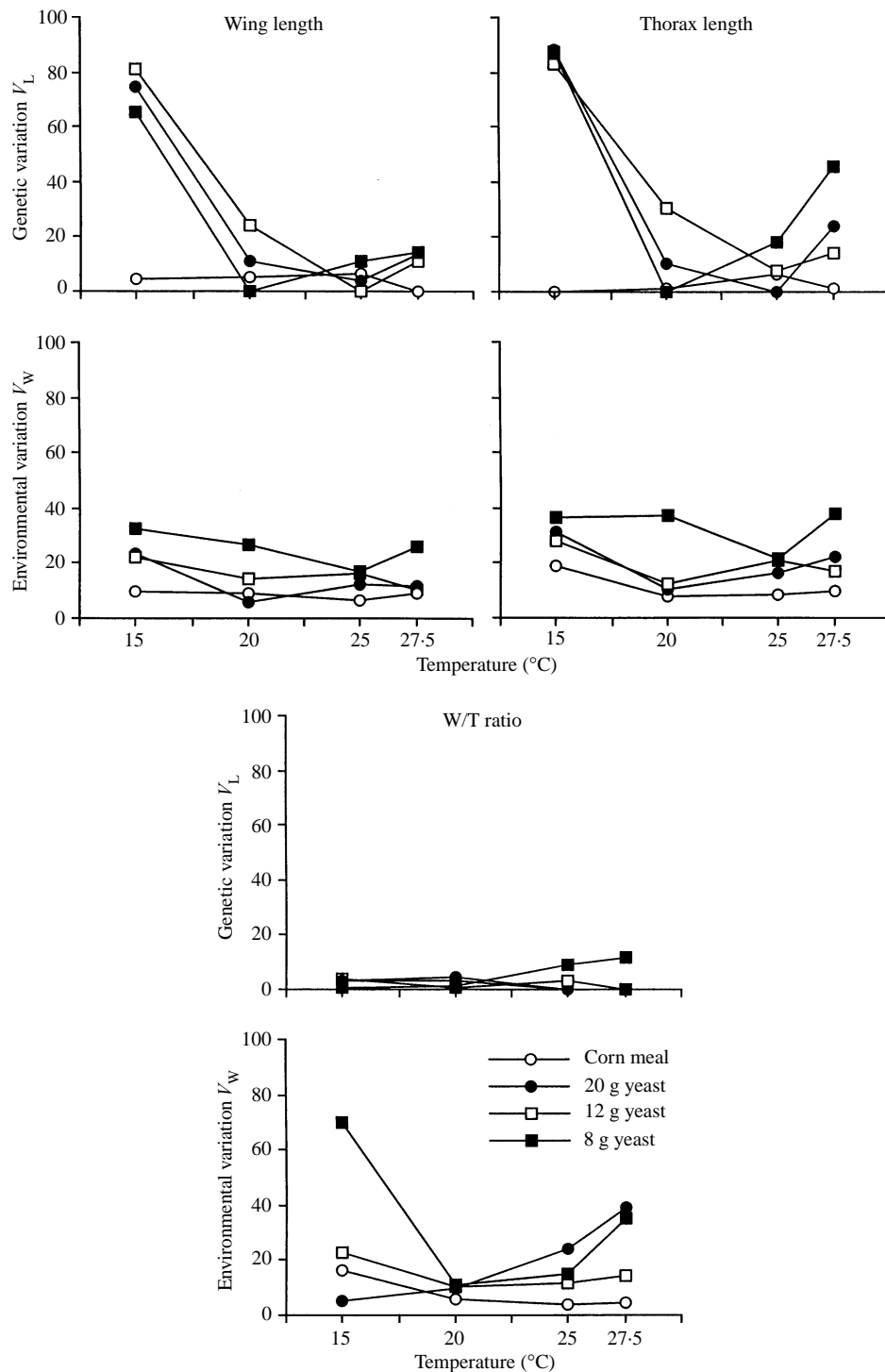
Fig. 3. Estimated levels of between-line variance (V_{L}) and within-line variance (V_{W}) for wing length, thorax length and the wing/thorax ratio at different temperature and food conditions. Error bars represent the 12·5–87·5 % confidence intervals, approximating 1 standard error.

relation between wing length and thorax length was disturbed under more stressful feeding conditions, leading to higher levels of V_{W} in the W/T ratio.

(iii) *Cellular basis of wing size response*

Under optimal food conditions, cell size showed a linear decreasing response to temperature. This re-

sponse levelled off at low temperatures under food-limited conditions (Fig. 4 a), similar to the response of wing length (Fig. 1 a). Reduced food conditions led to a reduction in cell size, with a much stronger response at low temperatures. However, the response of cell size could not fully explain the response of wing size to temperature and to food conditions. Both a reduction in food level and an increase in temperature
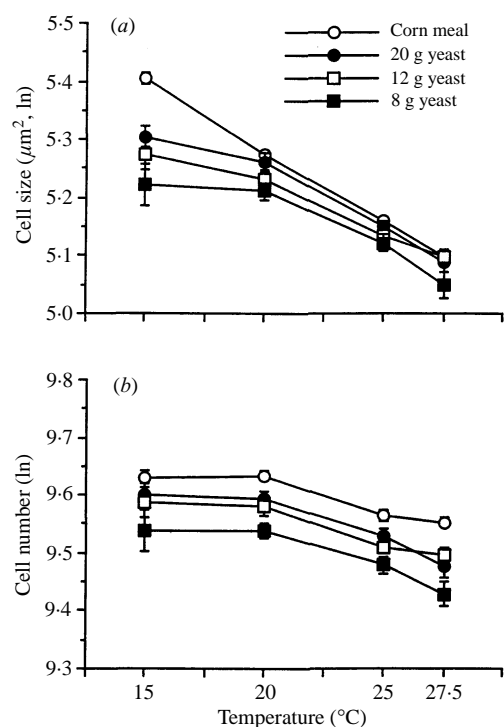
Fig. 4. Mean cell size (*a*) and cell number (*b*) ($\pm$SEM) in relation to temperature and food level. The standard errors may be too small to appear.

led to a reduction in cell number (Fig. 4*b*). Unlike cell size, cell number did not show a significant interaction between food level and temperature (Table 2).

ANOVA showed that lines differed significantly in both cell size and cell number (Table 2). The significant Temperature × Line and Food × Line interactions of cell size and cell number showed that their reaction norms differ in shape in their response to the two environmental factors.

The amounts of between-line variation V_L and within-line variation V_W of cell size and cell number

are shown in Fig. 5. The strong interaction effect of temperature and food level on V_L, observed in wing length, was much less pronounced in cell size. Under poor food and low temperature conditions V_L was lower for cell size than for wing length, while V_L is more or less similar at the other environmental conditions. Clearly, variation in cell size could not fully explain the patterns of variation in wing length. The increase in wing size variation under low temperature and poor food conditions must have been partly due to an increase in the variation in cell number (Fig. 5).

V_W of cell size and cell number were largely unaffected by the environmental conditions. Only at the lowest temperature and food level was V_W in cell size somewhat larger. The increase in V_W of wing length with the reduction in food level (Fig. 3) could not be attributed to one of its cellular components, but appears to be due to an increase in V_W of both cell size and cell number.

The interrelation between cell size, cell number and wing size over environments was analysed by plotting mean cell size and cell number per temperature and food level combination for each line separately (Fig. 6). Cell size and cell number were positively correlated over environments. Because both cell size and cell number are log-transformed, all cell size/cell number combinations that lead to the same wing size are connected by a straight line with slope −1, with larger wing sizes given by a parallel line with a higher intercept (Fig. 6).

The logarithmic cell size/cell number trajectories were specific for each line. In the trajectories, temperature variation and food level variation are not distinct, pointing to overall regulation of wing size. Visual examination of Fig. 6 would suggest some curvilinearity, but a departure from linearity could not be shown. The slope of the regression lines of cell

Table 2. *Results of ANOVA on* (*a*) *cell size and* (*b*) *cell number, using a mixed model design*

Source	d.f.	(a) Cell size		(b) Cell number	
		MS	F	MS	F
Environment					
Temperature (T)	3	77·90	86·94***	20·9	35·58***
Food (F)	3	10·10	25·99***	14·2	41·77***
T × F	9	1·93	6·82***	0·34	1·46
Genotype					
Line	7	3·59	16·18***	5·41	37·21***
L × T	21	0·89	4·03***	0·58	4·04***
L × F	21	0·39	1·75*	0·34	2·34***
L × T × F	63	0·28	1·28	0·24	1·63**
Error	181	0·22		0·15	
Total	309	1·27		0·67	

From Sokal & Rohlf (1981).
Both characters are log-transformed.
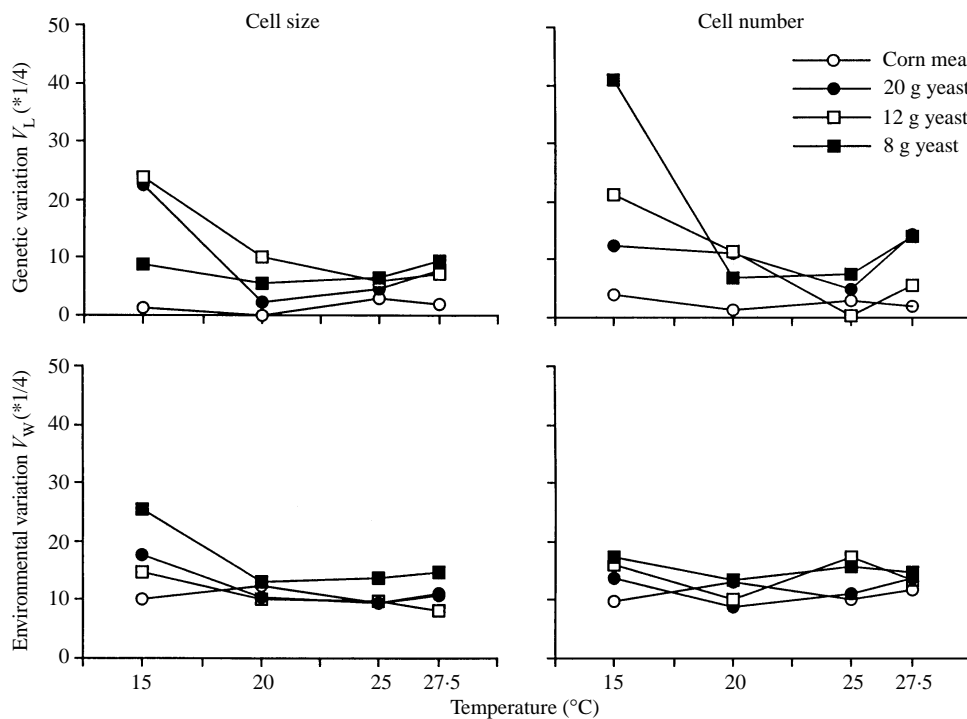* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 5. Estimated levels of between-line variance (V_L) and within-line variance (V_W) for cell size and cell number at different temperature and food conditions. To correct for the difference in dimension with wing length, V_L and V_W of (log-transformed) cell size and cell number are divided by four. Error bars represent the 12·5–87·5 % confidence intervals, approaching 1 standard error.
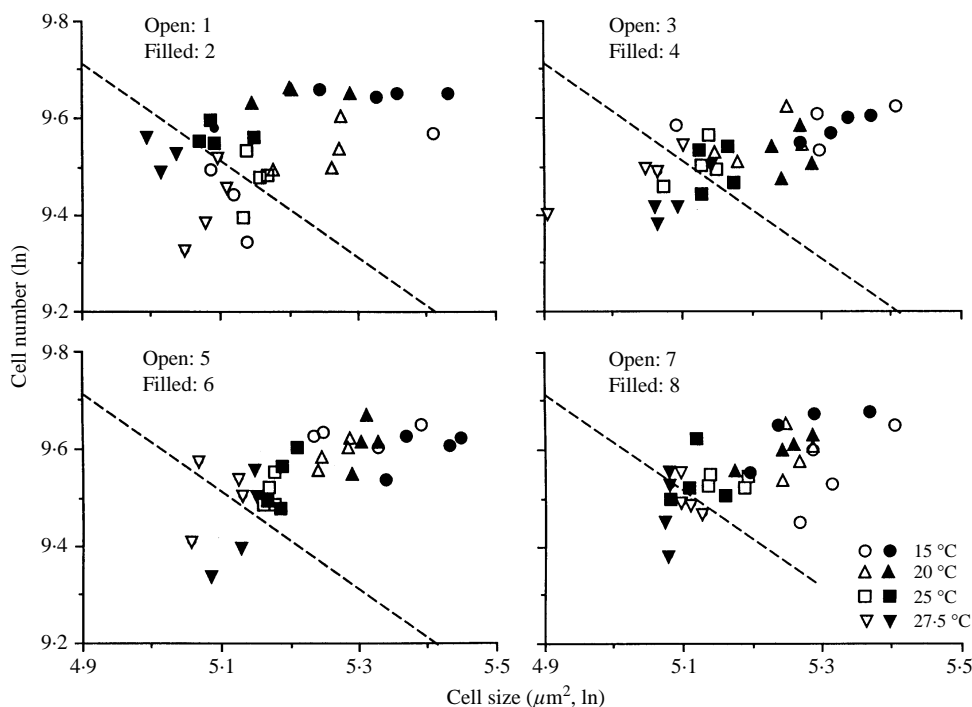

Fig. 6. Mean cell size and mean cell number per temperature at four food levels for the lines 1 to 8. The dashed line indicates equal wing sizes of ln(wing area) = 0·80.

number on cell size varied from 0·286 to 0·604; all slopes differed significantly from zero. Analysis of covariance showed a significant difference in overall level of cell number, taking cell size as a covariable (Line: $F_{7,299} = 16·72$, $P < 0·001$), and a highly signifi-

cant overall regression of cell number on cell size of $0·414 \pm 0·031$.

Over all trajectories together a difference between the small wing, high temperature area of the trajectories and the large wing, low temperature area

appears. The average cell size and cell number for each line were computed per temperature. The slope of the reduced major axis of the residuals increases with temperature ($b_{rma} \pm$ SE: 15 °C, 0·697 (0·535–0·919); 20 °C, 1·135 (0·845–1·524); 25 °C, 1·724 (0·871–3·420); 27·5 °C, 2·008 (1·472–2·739)). This implies that the more important changes at small wing sizes are in cell number, while the more important changes at large wing size are in cell size.

4. Discussion

Temperature and food level interact in determining the reaction norms of body size in *Drosophila melanogaster* and interact in determining the genetic variation in body size and its components. Using either environmental variable separately does not necessarily provide a clear view of the potential genetic differences in body size. The cellular basis of wing size changes in response to the environment seem to be line-dependent rather than determined by the environmental factor. Assigning cell size changes to temperature and cell number changes to food limitation might be an experimental artifact.

(i) *Environmental effects on genetic variation*

Under optimal food conditions, we observe little effect of temperature on the amount of expressed genetic variation V_G in wing length and thorax length in *D. melanogaster*. Similar results were obtained for other European populations of *D. melanogaster* and *D. simulans* (David *et al.*, 1994; Noach *et al.*, 1996; Morin *et al.*, 1996). A slight increase in V_G was observed only at temperatures below 17 °C (David *et al.*, 1994). These data seem to indicate that in *Drosophila*, under optimal food conditions, expressed genetic variation in wing length and thorax length mainly increases near the limits of its temperature tolerance range.

A reduction in food does lead to an increase in V_G in wing length and thorax length. Gebhardt & Stearns (1988, 1993) observed in *D. mercatorum* and *D. melanogaster* an increase in between-line variability of body weight at reduced food levels. Our data show a sharp increase in V_G in food-limited conditions, but mainly at the lowest temperature tested. These observations are in line with the hypothesis of Hoffmann & Parsons (1991) that stressful conditions generally lead to an increase in expressed genetic variation.

However, the V_G in wing to thorax (W/T) ratio showed a sharply deviating pattern. V_G is extremely low and scarcely depends upon environmental conditions. The consistency of genetic differences over environments indicates that the response of the W/T ratio to temperature is highly regulated, which indicates a strong relation between the W/T ratio and fitness (David *et al.*, 1994).

More extreme environments also lead to higher levels of V_E. David *et al.* (1994) and Morin *et al.* (1996) observed an increased within-line variation at extreme temperatures. We observed no such effect of temperature on V_E, possibly due to the smaller temperature range used in this experiment. However, we did find a clear effect of food level on V_E at each temperature. Under more extreme conditions, individuals might be more sensitive to small differences in food conditions.

We do not regard the increase in V_G in more extreme environments as a consequence of inbreeding depression. No evidence of inbreeding depression was observed, i.e. any decrease in viability was absent. The isofemale lines will differ in allele frequencies from the original population, and overall an increase in homozygosity will have taken place. However, it has long been known that very high inbreeding coefficients are necessary before any effect of inbreeding on quantitative characters occurs (Tantawy & Reeve, 1956). We interpret the increase in between-line variance in more extreme environments as representing the genetic variation in the original population under similar circumstances. Genotype by environment interaction in reaction norms might well lead to such a pattern.

(ii) *Cellular basis of wing size variation*

When plotting cell number against cell size, the response to temperature and food level largely follows a trajectory that is characteristic for each line (Fig. 6). The trajectory varies between lines in slope, that is, in the way wings of identical size are composed in terms of cell number and cell size. The main difference between lines appears to be an overall difference in cell number, especially at larger wing sizes. Similarly, earlier observations indicate that populations differing in wing size when raised under optimal food conditions at 25 °C, mainly differ in cell number (Robertson, 1959; James *et al.*, 1995).

Earlier studies observed that the effect of temperature on wing size is mainly based on changes in cell size (Alpatov, 1930; Robertson, 1959; Masry & Robertson, 1979; Partridge *et al.*, 1994), while a reduction in food level (Robertson, 1959) or crowding (Alpatov, 1930) leads to a reduction in cell number and, to a lesser extent, cell size. These results suggest an independent action of temperature and food level on the cellular components of wing size. In contrast, this study suggests that the two environmental factors attain their effect on wing size largely by a common mechanism, following a line-specific trajectory of cell size and cell number. The difference in effect of temperature and food observed in earlier studies appears to be due to a difference in size range of the observed flies. While the effect of temperature was studied at optimal food conditions, generating relatively large wing sizes, the effect of food was studied at

25 °C, at which temperature wing size is relatively small and the reduced major axis of the residuals indicates a predominant effect of cell number. The conclusion from earlier studies that the two environmental factors show a distinct cellular response, with temperature changes leading mainly to changes in cell size and food level reduction leading mainly to a reduction in cell number, seems to be due to the specific set of environmental conditions chosen, as indicated by the change in reduced major axis of the residuals within a temperature.

Temperature and food level interact in determining wing size. However, the way changes in wing size are accomplished by changing cell size or cell number appears to be largely independent of the environmental factor causing the change in wing size, but rather seem to be line specific. Therefore, it is the developmental relation between cell size and cell number that is under developmental control within each line, rather than cell size or cell number separately.

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