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The contrasting genetic architecture of wing size and shape in *Drosophila melanogaster*

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Surprisingly little is known about the genetic architecture of body size in natural populations of *Drosophila melanogaster*. Using both generation means and triple-test-cross analyses, we investigated the genetic architecture of wing size (an indicator of body size) and wing shape in a naturally occurring body size cline. For wing size, we found significant epistatic genetic variance and evidence of past directional selection for increased body size. While wing shape also exhibits significant epistatic genetic variance, there was no indication of directional selection, suggesting instead a history of optimizing selection. Our results support the idea that epistatic variance may be more common in natural populations than was once suspected. Also, our results suggest substantial directional selection on wing size but not shape.

Keywords: directional selection, *Drosophila melanogaster*, epistasis, genetic architecture, wing shape, wing size.

Introduction

Body size is of central importance in evolution and ecology, and it has been study extensively in both artificial and natural environments. Numerous allometric relationships between life history, physiological and behavioural traits and body size have been reported across species (e.g. Schmidt-Nielsen, 1984). Various trade-offs affecting body size have been identified (Stearns, 1992). Although artificial selection experiments provide numerous insights into aspects of the evolution of body size, the generality of the findings is necessarily limited. Attempts to understand the how and why of evolution must eventually involve studies of natural populations.

In *Drosophila*, in particular, surprisingly little is known about the quantitative genetics of body size of natural populations, apart from its high heritability (Coyne & Beecham, 1987; Prout & Barker, 1989; Ruiz *et al*., 1991; Thomas & Barker, 1993). Our intention in this study was to answer some additional and important questions regarding the evolution of body size in *Drosophila melanogaster* in a natural body-size cline. First, using wing area as a measure of body size, we questioned the importance of epistasis in population divergence. The answer is relevant not only to the shifting balance theory of evolution, but also to questions regarding the evolution of mating systems and conservation genetics (see Whitlock *et al*., 1995; Fenster *et al*., 1997). Secondly, what sort of selection (either directional or optimizing) is likely to be the predominant form of natural selection acting on the trait? Body size in *D. melanogaster* has an intermediate optimum value, largely determined by correlations with fecundity, development time and larval survival (Roff, 1981). Thus, although appearing to be under optimizing selection, the optimizing selection on body size is only apparent (Falconer, 1989): it is not clear what type of selection acts directly on genes determining body size within the constraints imposed by correlated characters.

In addition to wing area, we also analysed wing shape. Although a number of investigators have documented natural variation in wing shape, little is known about the evolutionary genetics of wing shape within species. Weber (1990) found evidence consistent with optimizing selection on wing shape in natural populations, while Bitner-Mathé & Klaczko (1999) found high heritability for shape in *D. mediopunctata*. Despite recent advances in the understanding of the aerodynamics of the wings of smaller insects (Lehmann & Dickinson, 1998), virtually nothing is known about the functional implications of variation of wing morphology (Grodnitsky, 1999). This makes any inferences about the action of natural selection on shape difficult. Further investigation into

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the genetics and selective forces affecting shape would help to determinine whether or not natural variation is adaptive.

In a previous study, we investigated clinal variation in wing area using a generation means analysis (Gilchrist & Partridge, 1999). In the present work, we have extended our earlier study, using both a triple-test-cross analysis and a generation means analysis to investigate clinal variation in wing shape as well as wing size. Together, these designs allow the additive, dominance and epistatic components affecting the traits to be estimated. Using this information, we can characterize the genetic architecture of the traits and draw inferences about the type of natural selection that has acted on the traits.

Methods

Flies

Parental lines were derived from populations sampled from extreme ends of the eastern Australian body-size cline (James *et al*., 1995). The smaller body-size, northern population came from Innisfail, Queensland (Inn, 17.30°S), while the larger body size, southern populations came from Cygnet, Tasmania (Cyg, 43.08°S). Both populations were sampled in January 1997 and maintained as outbred bottle populations. All flies used for measurement were picked as first instar larvae and raised in vials of unyeasted standard medium, at a constant density (30 larvae per vial), conditions under which competition is minimal and body size maximized. All flies emerging from each vial were frozen for later measurement.

A number of isogenic lines, derived from the Cyg and Inn populations, were constructed in late 1997 in a two-stage process to avoid problems associated with low-level recombination when the three major chromosomes are balanced simultaneously. First, individual X-chromosomes were homogenized using an FM7 balancer stock. Secondly, pairs of second and third chromosomes were homogenized using a SM5/bw^{V1}; TM3/TM6B stock. Separate X-isogenic lines and II-and III-isogenic lines were subsequently combined to produce lines that were isogenic for all three major chromosomes. Both isogenic lines used in the present experiments were smaller (although not significantly so) than the outbred stock from which they were derived. This is consistent with low values of inbreeding depression for body size within populations of *D. melanogaster* (Tantaway, 1957; Fowler & Whitlock, 1999). The smaller isogenic line was approximately 82% the size of the larger line, the same relative difference that exists in the outbred stocks (approximately 84%).

Measurements

We used wing area as an estimate of body size, because the two characters are highly correlated (Reeve & Robertson, 1952). Wing measurements were performed as detailed in Gilchrist & Partridge (1999). Briefly, wing images (one per fly) were captured using a compound microscope-mounted video camera. Using the OBJECT-IMAGE program (Vischer, 1998), coordinates of 10 wing landmarks were recorded and the wing area calculated based on the polygon shown in Fig. 1. From the same landmark data we also extracted the principle components (PCs) of shape variation for use as shape variables. PCs were computed using covariance matrices after the landmark data had undergone a Procrustes superimposition, a process of reflection, scaling (using centroid size) and rotation that produced a superimposition minimizing deviations from the overall mean shape. The process is described in detail in Dryden & Mardia (1998).

Experimental designs

We measured the genetic components of both wing size and shape using two complementary biometrical designs. The first, a generation means analysis, was performed on the cross between two extreme populations from a natural body-size cline. This analysis estimated net genetic effects on the mean phenotypic values of the hybrid generations (as detailed in Kearsey & Pooni, 1996). The second experimental design, the triple-test-cross (TTC) design, analysed genetic variance components, rather than means, in the cross between isogenic lines from the cline ends (Kearsey & Jinks, 1968; Kearsey, 1980). These two approaches (i.e. a means analysis and a variance analysis) are complementary

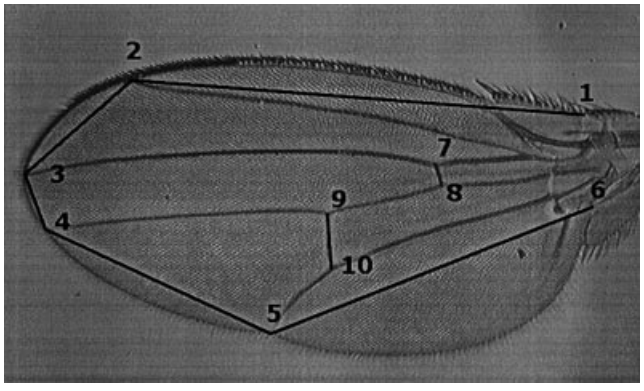

Fig. 1 The landmarks used as the basis of both wing size and shape measurements. Wing size was calculated using the landmarks 1–6, while shape calculations also included the four crossvein landmarks.

because they measure different aspects of underlying gene action and interaction (Jinks, 1979; Fenster *et al*., 1997). As the means analysis measures net genetic effects on means while the TTC measures variance components, their parameters are not correlated. Thus, for example, when the effects of increasing and decreasing dominant alleles are spread evenly between the two populations, the means analysis will show a zero net dominance (i.e. ambidirectional dominance), whereas a TTC analysis may detect significant dominance variance (because the variance is unaffected by the net direction of dominance). Alternatively, when dominance is directional rather than ambidirectional, the means analysis has the advantage of showing the direction of the dominance, something that cannot be inferred easily from the variance analysis. Similarly, the means analysis may detect epistatic interactions near fixation, while the variance resulting from the same alleles may be very small. Either analysis taken alone may produce an ambiguous indication of the genetic architecture, but together provide a clearer picture on the genetic architecture of the trait.

Generation means analysis

This analysis was performed on data from Gilchrist & Partridge (1999), where models describing wing size, but not wing shape, were presented. The generation means analysed were the two parental populations and their F_1, F_2 and back-cross generations. Keeping reciprocals separate, 14 generations were raised and wing traits measured. Using a weighted least-squares analysis (Kearsey & Pooni, 1996; Lynch & Walsh, 1998), composite additive ([a]), dominance ([d]), digenic epistatic ([aa], [ad] and [dd]) and maternal effects were calculated. Goodness-of-fit tests (using χ^2-values) were then used to determine the model (incorporating some or all of these composite effects) that best described the observed generation means. Changes made to the method used in Gilchrist & Partridge (1999) involved a new parameter accounting for the additive effect of the X chromosome, [a_X], that differs between male and females. In addition, the sampling variance was calculated separately for each generation, accounting for possible effects of variation in within-generation variance for different genotypes (Robertson & Reeve, 1953).

For the shape analysis, PC1 and PC2 were extracted from the two outbred parental populations. These provided our basic shape variables, capturing over 40% of the shape variation between the cline end populations (Table 1). Figure 2 shows the shape change associated with PC1 and PC2. PC1 describes a relative enlargement of the posterior region of the wing coupled with displacement of the posterior crossvein. PC2 describes an arching of the wing along the longitudinal axis (*D. melanogaster* wings appear to have very little affine shape variation). For both the means analysis and the TTC, shape measurements were collected by projecting the Procrustes co-ordinates of the hybrid generations onto the parental PC axes. Strictly speaking, PCs are valid only for the populations from which they were derived and are not expected to be orthogonal in other groups. However, we were not interested in distinguishing between PC1 and PC2; instead we regarded PC1 and PC2 as different aspects of a single character (i.e. shape). Therefore, their independence was not crucial to our analysis. Nevertheless, when the Procrustes co-ordinates of the hybrid generations are projected onto the parental PC axes, PC1 and PC2 remain uncorrelated ($r = -0.040$,

Table 1 The percentage variance accounted for by shape principal components 1–5

PC	Females	Males
1	23.9	23.4
2	18.7	18.7
3	15.3	14.4
4	10.8	10.8
5	9.3	8.4

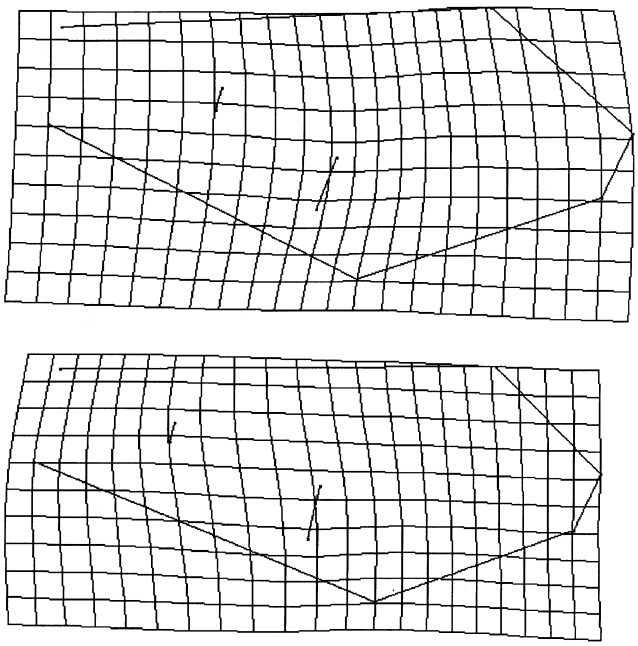

Fig. 2 The transformation grids describing the shape variation measured for female wings. The shape variation shown represents the distortions necessary to move from the larger parental wing shape to the smaller parental wing shape for PC1 (upper) and PC2 (lower). The reference points are the same as those indicated in Fig. 1.

$P = 0.07$). Also, the variance of the landmarks did not significantly differ between the parental and hybrid populations. Therefore, PC1 and PC2 were adopted as two distinct, but not necessarily independent shape measures.

TTC analysis (variance analysis)

Individual F_2 males were crossed sequentially to groups of six P1, P2 or F_1 females (the three test crosses). All test crosses to the same tester (P1, P2 or F_1) were performed on the same day and all larvae reared on the same batch of food medium. In practice, the progeny production of these crosses was very poor. As a result, many crosses failed to produce sufficient progeny for measurement, while the low numbers of progeny generally precluded replication of vials. Only data from F_2 males that produced at least eight measured individuals of either sex in all three test crosses were used. This allowed the data to be analysed as a completely balanced two-way ANOVA, simplifying the partitioning of mean squares. The TTC analysis provides a test for epistatic variance which was partitioned into a V_{aa} component and a combined V_{ad}/V_{dd} component (using the method set out in Kearsey & Pooni, 1996). Parameters of the variance analysis are indicated as V_a, V_d, V_{aa}, etc.

The outbred parental PC axes were also used for the TTC analysis. The Procrustes transformed TTC landmarks were rotated so that the mean co-ordinates were the same as those of the Procrustes transformed outbred parental landmarks. The Procrustes transformed TTC landmarks were then projected onto the parental PC axes. The result of this procedure is that the TTC tested for the same pattern of shape variation that was measured in the outbred populations. The raw shape variation between the parental isogenic lines represents a biased sample of the shape variation between the outbred populations. Therefore, if we had used PCs extracted directly from those data, in effect, we would have been analysing a different shape character. Because we were interested only in the outbred shape variation and not a biased sample, we again considered that projection onto the outbred parental PC axes was justified. Although PC1 and PC2 were significantly correlated in the TTC (females, $r = 0.16$; males, $r = -0.12$), their independence was not crucial to our analysis.

Software

Full Procrustes superimpositions were performed using the programs MORPHOMETRIKA 007 (Walker, 1998) and MORPHOLOGIKA 1.1 (O'Higgins & Jones, 1999). Statistical analyses were performed using JMP 3.2.2 for the Macintosh (SAS Institute, 1994). In order to visualize the shape changes attributable to each source of variation, we used MORPHOLOGIKA 1.1 (O'Higgins & Jones, 1999), which allowed the effects of continuous variation in a single PC to be visualized using wireframe models while other PCs were be held at zero.

Results

Wing size

Area data were transformed by taking natural logs. This transformation produced a slightly lower epistatic variance and eliminated a small increase of variance with size (in the males only). The results of the generation means analysis are shown in Table 2. As expected, the significant parameters are the same as those presented in Gilchrist & Partridge (1999), despite the data being transformed and the use of additional parameters. For the females, the additive effects were both autosomal and X-linked. There was net dominance for increasing alleles. The [ad] component was also reflected in the TTC analysis (Table 3), where the combined V_{ad}/V_{dd} component was highly significant. The model describing male wing area was similar, with directional dominance for larger wings and significant [ad] interaction. Analysing the data as a North Carolina III design (in which F_2 individuals are backcrossed to both parents, but not to the F_1 tester; Kearsey & Pooni, 1996), estimates of V_a and V_d were obtained (Tables 4 and 5). Although these estimates were biased by the presence of epistatic variance, they indicate significant V_d for wing area in both sexes, as expected on the basis of the significant [d] in the means analysis. The significant directional dominance for larger wings and large amounts of interaction in both sexes are indicative of past directional selection on wing area.

Wing shape

Our basic shape variables were PC1 and PC2, calculated separately for each sex. The only significant correlation with wing area occurred in females for PC2 ($r = 0.35_{[281]}$, $P < 0.001$). This allometric variation could be the result of two possible underlying causes: (i) an underlying developmental constraint, i.e. the genetic mechanism controlling shape imposing particular shape variation as wing size increases; or (ii) parallel selection with wing area. If PC2 represents a developmental constraint, we have no expectation for its genetic architecture. If it is selected with size, then PC2 may have a similar genetic architecture resembling that of wing area. PC1, however, was not consistently correlated with size. Accordingly, it is unlikely to be subject either to developmental

Table 2 The estimates of composite genetic effects affecting mean wing area (ln transformed) and wing shape (PC1 and PC2). Subscript X denotes the X chromosome and subscript M denotes a maternal effect. Note the absence of dominance effects from the PC models

	Females ($\times 10^4$)			Males ($\times 10^4$)		
	ln(WA)	PC1	PC2	ln(WA)	PC1	PC2
m	2443.6	−5.6	21.8	−205.3	−22.6	5.0
[a]	728.1***	11.6**		803.9***		20.5***
[a_X]			43.7***		−18.1***	
[d]	219.3***			127.3*		
[a.a]			−36.5***		19.6**	
[a_X.a_X]		−15.7***	17.3***			
[a.d]	−329.7***			−460.9***		
[a_m]					−36.5***	
[d_m]	−189.7***	9.3*		−208.7***		14.1***
[c]			−12.5***	−86.8		
Y					11.3***	−6.2**
χ^2	18.73*	29.23**	41.52***	25.31**	40.95***	41.87***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3 Triple test cross analysis of variance for wing area (ln transformed) and wing shape (PC1 and PC2), showing total epistasis partitioned between V_{aa} and V_{ad}/V_{dd}

		ln(WA)		PC1		PC2	
	d.f.	MS	*F*	MS ($\times 10^4$)	*F*	MS ($\times 10^4$)	*F*
Females							
V_{aa}	1	0.0151	2.78 NS	3.009	2.84 NS	0.592	< 1 ns
V_{ad}, V_{dd}	32	0.0051	2.42***	0.998	1.45 NS	1.949	2.31***
All epistasis	33	0.0054	2.57***	1.059	1.53 *	1.908	2.26***
within FS families	759	0.0021		0.690		0.844	
Males							
V_{aa}	1	0.0719	9.91***	0.410	< 1 NS	1.212	< 1 ns
V_{ad}, V_{dd}	36	0.0055	2.57***	0.851	1.40 NS	2.050	2.86***
All epistasis	37	0.0073	3.42***	0.839	1.38 NS	2.027	2.83***
within FS families	851	0.0021		0.609		0.717	

NS, not significant ($P > 0.05$); * $P < 0.05$; *** $P < 0.001$.

Table 4 Analysis of variance for PC1 data when treated as a North Carolina III design

	Females			Males		
	d.f.	MS ($\times 10^4$)	F	d.f.	MS ($\times 10^4$)	*F*
Testers	1	34.8	30.85***	1	217.3	350.9***
F2s (additive)	32	3.99	7.45***	36	2.56	5.62***
TxF2s (dominance)	32	1.13	2.11***	36	0.619	1.36 NS
within FS families	528	0.535		592	0.456	

NS, not significant ($P > 0.05$); *** $P < 0.001$.

constraint or size-related selection. PC1 may represent a shape component free to respond to natural selection.

For both PC1 and PC2, the means analysis provided no sufficient model for either sex (Table 2), indicating the presence of higher order interactions and/or linkage. In both sexes there were significant [a], [a_x], [aa] and [$a_x a_x$] components and maternal effects. In contrast to wing area, X-linked alleles appear to have a much larger

Table 5 Estimates of additive and dominance variance for the three traits indicated. Significance was calculated following (Kearsey, 1980)

	Wing area		PC1		PC2	
	Females	Males	Females	Males	Females	Males
V_a* ($\times 10^4$)	33.4***	31.1***	0.86***	0.53***	0.73**	1.03**
V_d* ($\times 10^4$)	4.1**	5.2**	0.07*	0.02 NS	0.09*	0.02 NS
h^2	0.81	0.75	0.71	0.61	0.61	0.80

NS, not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

effect on shape. Notably, there were no significant [d] or [dd] effects apparent for either PC in either sex. In the variance analysis (Table 3), PC1 showed significant epistatic variance only in females. The partitioning did not detect the predominant form of epistatic variance, and therefore the significant [aa] effects detected in the means analysis were not reflected in the variances. A possible explanation for this discrepancy is that the additive-by-additive component is the consequence of a large number of individually small interactions between many genes, a situation that can result in significant [aa] but nonsignificant V_{aa} (Kearsey & Pooni, 1996). Using a North Carolina Design III (shown for PC1 in Table 4), estimates of V_a and V_d were obtained as for wing area (Table 5). In the case of the males, these estimates were unbiased because there was no significant epistasis, while the female estimates were biased to some degree by the small amount of epistatic variance. V_d for PC1 was not significant in males and V_d was small and only marginally significant in females. Given that dominance for PC1 is either small or absent, the genetic architecture of PC1 is predominantly additive, suggesting a history of optimizing rather than directional selection. In both sexes, the heritability of PC1 was high (Table 5).

Estimates of V_a and V_d were also obtained for PC2, although these estimates are necessarily biased because of the highly significant epistatic effects on the variances (Tables 3 and 5). For PC2, male V_d was again not significantly different from zero, while for females V_d was again small but significant ($P = 0.04$). This suggests that, for males at least, the absence of dominance effects on the means was in fact because of the absence of dominance variance, not ambidirectional dominance. Again, the low levels of dominance variance are suggestive of optimizing rather than directional selection on wing shape.

Discussion

Using a biometrical analysis of both generation means and variances of wing size and shape we have shown, first, that the wing size divergence of *D. melanogaster* populations in the eastern Australia cline involves significant epistatic variance. Secondly, in comparing the genetic architectures of wing area and wing shape we have shown that, while size is likely to have been subject to directional selection, shape is likely to have been subject to optimizing selection.

Interest in epistasis is founded squarely on its role in Wright's shifting balance theory (Wright, 1977). Epistasis is proposed to have a vital role in shaping multiple fitness peaks for interbreeding populations. Ideally, the existence of multiple peaks could be established simply by measuring epistasis for fitness. However, such direct measurement is extremely difficult, if not impossible (Whitlock *et al*., 1995). Instead, measurement of the role of epistasis in characters that are both more amenable to measurement and closely related to fitness produces circumstantial evidence for the importance of epistasis in shaping fitness profiles. In *D. melanogaster*, similar natural clines in wing area exist on most continents, suggesting that wing area must be highly correlated with fitness. Our results demonstrate that genes determining wing area show significant amounts of epistatic interaction and that there is also significant epistatic genetic variance. Previous results have shown that the epistatic parameters (determined only from a means analysis) vary considerably between continents (Gilchrist & Partridge, 1999). This variety implies that different genetic combinations can generate similar size divergence, involving either different alleles or the same alleles at different frequencies. The presence of significant amounts of epistatic genetic variance suggests that the evolutionary response to size selection may involve selection of alternate combinations of interacting alleles. The genetic landscape for wing area may have many alternate peaks. The relevance of this result lies in the degree to which epistasis for size corresponds to epistasis for fitness. There is evidence of correlations between additive chromosomal effects on size and fertility, but correlations between interaction terms for size and fitness are much harder to demonstrate (e.g. Cavicchi *et al*., 1989).

The second point to emerge from our results concerns the evolutionary history of different aspects of wing morphology. The predominant form of selection affecting the traits was inferred from the genetic architecture of both wing size and shape. Different forms of selection, either directional or optimizing, operating over sufficiently long periods are expected to produce

recognizable patterns in underlying genetic parameters. A view formulated by Mather, and originally based on Fisher's ideas of the evolution of dominance, is that directional selection should result in relatively larger dominance components for fitness traits than for morphological traits (e.g. Mather, 1966; Mather, 1983). Although the evolution of dominance as originally envisaged is open to serious question (Orr, 1991), a number of studies have shown patterns of genetic architecture consistent with the original expectation, i.e. higher dominance variance for directionally selected traits (e.g. Breese & Mather, 1960; Kearsey & Kojima, 1967; Kearsey & Barnes, 1970). A likely cause of increased dominance in fitness traits is that alleles with positive effects on fitness will be selected, regardless of their degree of dominance. The erosion of additive genetic variance under directional selection is also expected to increase the relative amount of dominance variance (Crnokrak & Roff, 1995), while selection on dominance at other alleles may occur in some circumstances (Mayo & Burger, 1997). An additional expectation is that duplicate epistasis should also arise in directionally selected traits, again to moderate the effects of new unfavourable alleles. By contrast, traits under optimizing selection are expected to have a predominantly additive architecture, with less pronounced dominance components. Because these traits have an intermediate optimum, alleles with dominance that move the trait mean in either direction away from the optimum will have equivalent, detrimental effects on fitness. Therefore, dominance will not be favoured and will be either reduced, absent or ambidirectional.

For body size, previous results have shown that *D. melanogaster* has a predominantly additive architecture, indicating optimizing selection (Kearsey & Kojima, 1967). However, these results were observed in crosses between laboratory strains, chosen without regard for size. Because we deliberately selected populations showing extreme body size divergence, these results are not strictly comparable. Our results suggest that the divergence of the Cyg and Inn populations has involved selection for dominant increasing alleles. At a given point along the cline, optimal body size would be determined by the balance of correlated characters, but the presence of directional dominance for body size would ensure a higher proportion of individuals were at the maximum possible body size permitted by the pattern of correlated characters. This may be particularly advantageous at lower temperatures, as indicated by the finding that genetically larger flies may have increased survival and lifetime fecundity at cooler temperatures (McCabe & Partridge, 1997).

Shape, however, is more complex. Although many studies have documented shape change in a variety of Drosophila species (Alonso & Munoz, 1984; Cavicchi *et al*., 1991; Bitner-Mathé *et al.,* 1995; Imasheva *et al*., 1995; Bublii *et al*., 1996; Pezzoli *et al*., 1997; Baylac & Penin, 1998; Haas & Tolley, 1998; Huey *et al*. 2000), there is little evidence indicating that natural shape change is adaptive. Because the general wing structure of five longitudinal veins and three cross-veins is common to the entire family Drosophilidae (Wheeler, 1981), shape might be thought to be highly canalized. Also, allometric shape variation is a common feature of most morphological structures. Therefore, shape variation may be limited by phylogenetic constraints and (assuming that the allometric shape variation is not selected) a significant portion of the observed phenotypic variation may be a consequence of developmental constraints (in the form of allometry). Any adaptive shape change would then involve only the remaining nonallometric shape component. While there are significant shape differences between populations of *D. melanogaster* at this level (Gilchrist *et al*. 2000), we lack even hypothetical functional explanations for such shape variation (Grodnitsky, 1999). For the present, there appears little chance of explaining observed shape variation in functional terms.

Instead of attempting a functional explanation, we have examined this variation from a biometrical viewpoint. On the basis of earlier results showing that the variation in shape, although significant, is small (Gilchrist *et al*. 2000), it was hypothesized that shape variation may simply represent drift around an optimum. Our results support this hypothesis. The genetic architecture of both PC1 and PC2 suggests an evolutionary history of optimizing rather than directional selection, implying an intermediate optimal phenotype. If small-scale shape variation is not adaptive, this optimal phenotype may instead be a product of developmental constraints or 'intrinsic' canalization (Gibson & Wagner, 2000). Canalization of the phenotype (i.e. reduced variation around the mean) may explain why the range of drift was so narrow, yet detectable. Our view of shape variation as a simple additive character, with little dominance or interaction, is consistent with other investigations of wing shape. Bitner-Mathé & Klaczko (1999) found that nonallometric PCs in *D. mediopunctata* have high heritability. A recent QTL analysis of artificially selected shape change found shape to be influenced by genes dispersed along the third chromosome, with nearly additive effects in heterozygotes (Weber *et al*., 1999). Two models were proposed to explain their results, both involving many genes (11 at least) with largely additive effects and either no or cancelling interactions. In a cross between two laboratory strains, Zimmerman *et al*. (2000) also found 34 possible shape QTLs with largely additive affects. If

shape is a simple additive character, then it may prove useful for the study of morphological variation. For example, once QTL intervals have been identified, candidate loci could be screened using complementation tests. However, in traits showing significant amounts of interaction and/or dominance, results can be ambiguous despite attempts to control for different genetic backgrounds by prior backcrossing (Gurganus *et al*., 1999). Where the effects of natural variation are entirely additive, complementation testing may be considerably easier.

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