doi:10.1111/j.1558-5646.2009.00774.x

PLASTICITY, CANALIZATION, AND DEVELOPMENTAL STABILITY OF THE *DROSOPHILA* WING: JOINT EFFECTS OF MUTATIONS AND DEVELOPMENTAL TEMPERATURE

Vincent Debat,[1,2] Allan Debelle,[1,3] and Ian Dworkin[4]

[1]*Muséum National d'Histoire Naturelle, UMR CNRS 5202, Département Systématique et Evolution, 45 rue Buffon, 75005 Paris, France*

[2]*E-mail: debat@mnhn.fr*

[4]*Department of Zoology, Program in Ecology, Evolutionary Biology, and Behavior, Michigan State University, East Lansing, MI 48824*

Received October 31, 2008
Accepted June 24, 2009

The phenotypic effects of genetic and environmental manipulations have been rarely investigated simultaneously. In addition to phenotypic plasticity, their effect on the amount and directions of genetic and phenotypic variation is of particular evolutionary importance because these constitute the material for natural selection. Here, we used heterozygous insertional mutations of 16 genes involved in the formation of the *Drosophila* wing. The flies were raised at two developmental temperatures (18°C and 28°C). Landmark-based geometric morphometrics was used to analyze the variation of the wing size and shape at different hierarchical levels: among genotypes and temperatures; among individuals within group; and fluctuating asymmetry (FA). Our results show that (1) the phenotypic effects of the mutations depend on temperature; (2) reciprocally, most mutations affect wing plasticity; (3) both temperature and mutations modify the levels of FA and of among individuals variation within lines. Remarkably, the patterns of shape FA seem unaffected by temperature whereas those associated with individual variation are systematically altered. By modifying the direction of available phenotypic variation, temperature might thus directly affect the potential for further evolution. It suggests as well that the developmental processes responsible for developmental stability and environmental canalization might be partially distinct.

KEY WORDS: **Canalization, *Drosophila* wing, fluctuating asymmetry, geometric morphometrics, phenotypic plasticity, variation.**

"*Evolution is the control of development by ecology*." Leigh Van Valen's famous aphorism is both illuminating and frustrating. Illuminating for that it captures in a single short sentence the essence of evolutionary developmental biology and frustrating because it points at an immediate problem: how can we practically integrate the complexity of ecology and development in evolutionary studies?

A global and systematic integration seems improbable in the general sense. However, a few cases have provided real successes of such an integration, such as the evolution of butterflies eyespots

[3]Present address: University of Sheffield, Department of Animal and Plant Sciences, Western Bank, Sheffield, S10 2TN, United Kingdom.

(e.g., Brunetti et al. 2001; Beldade and Brakefield 2002; Allen et al. 2008), the evolution of sticklebacks body armor (e.g., Peichel et al. 2001; McKinnon and Rundle 2002; Cresko et al. 2004; Colosimo et al. 2005; Marchinko and Schluter 2007; Barrett et al. 2008), or the evolution of the shade avoidance syndrome in *Arabidopsis* (Smith and Whitelam 1997; Callahan and Pigliucci 2002; McGuire and Agrawal 2005). Several other models have provided promising attempts (e.g., *Drosophila* pigmentation; Gompel et al. 2005; Gibert et al. 2007; Jeong et al. 2008; Williams et al. 2008).

If long-term research programs are needed to get a global picture of the ecology, developmental biology, and evolution of a group of organisms, a few concepts stands logically at this interface: such are phenotypic plasticity, evolvability, robustness, developmental stability, modularity—all related to phenotypic variation. This is because phenotypic variation is the raw material on which selection acts; and ecological factors interact with development to produce the phenotype and because phenotype is the end result of both evolution and ontogeny and its variation thus reflects changes in both development and evolution. Studying phenotypic variation is thus important to understand evolution, ecology, and development; but considering evolution, development, and ecology is necessary to understand phenotypic variation (e.g., Hallgrimsson and Hall 2005).

In this context understanding the nature of the processes involved in the control of phenotypic variation is of particular importance: the relationship between phenotypic plasticity—a phenotypic change in response to a change in environment; canalization—the ability to produce a consistent phenotype in spite of genetic and environmental influences; and developmental stability—the buffering of random developmental errors, has generated some debate (e.g., Debat and David 2001; Flatt 2005). Specifically, the link between developmental stability, as assessed by fluctuating asymmetry (FA) and canalization is controversial. Based on the comparison of the patterns of variation among individuals with those of FA, some authors, in line with the original work of Waddington (1942, 1957) have suggested that they are at least partly different processes (e.g., Debat et al. 2000; Milton et al. 2003; Pélabon et al. 2004; Rego et al. 2006). In turn, the view that there is no need for more than one single buffering mechanism has been defended by others (e.g., Clarke 1998; Klingenberg and McIntyre 1998; Willmore et al. 2005; Breuker et al. 2006).

The *Drosophila* wing provides a particularly amenable model phenotype to investigate the developmental control of variation in this framework. It is involved in several functions of ecological and evolutionary importance (e.g., flight and male courtship song) and its developmental genetics is understood in great detail (e.g., De Celis 2003). Additionally, *Drosophila* wing size and shape demonstrate plastic responses to temperature, which have been extensively investigated and have been suggested to be adaptive (e.g., David et al. 1994; Partridge et al. 1994; Imasheva et al. 2000; Gilchrist et al. 2004; David et al. 2006).

In this article, we report on the effects of a series of mutations in genes known for their role on the morphogenesis of the *Drosophila* wing, on the phenotypic response to different developmental temperatures.

Using geometric morphometrics (Rohlf and Marcus 1993), we examined variation for wing size and shape at three hierarchical levels: (1) among groups (i.e., the different genotypes placed in the different temperatures), (2) among individuals within a genotype (referred to as individual variation), and (3) within individuals (FA and directional asymmetry [DA]). This hierarchy allows testing, respectively, for genetic differences in phenotypic plasticity, in environmental canalization, and in developmental stability.

Material and Methods

EXPERIMENTAL SETUP

We compared the wings of wild-type and mutant flies reared in two thermal regimes. Sixteen different mutant genotypes were used consisting in heterozygous insertions of transposons within genes involved in the wing development. The detailed experimental procedure used to obtain the flies is described in Dworkin and Gibson (2006). The insertional mutations were selected from the Bloomington Stock Center (Table 1). All insertions were introgressed into an Oregon-R wild-type strain marked with *white* (*w*), resulting in white-eyed flies (Dworkin and Gibson 2006). The transposons used for the insertions were marked with a mini white element, rescuing the eye color defect. Flies carrying the insertions thus all had red eyes. Backcrossing of females carrying the mutation to wild-type males was repeated for 14 generations so that the mutant genetic background would be as close as possible to the isogenic wild type. The mutants were compared to Oregon-R wild-type individuals from separate vials. For each genotype (including the Oregon-R control), two sets of adult flies each composed of 10 virgin females and a few males were placed in two vials containing a standard medium. The flies were removed after two days, and the medium containing the eggs was subdivided into two replicate vials. These two vials per genotype were then placed in each of two incubators, respectively, set at 18°C and 28°C and regularly controlled for temperature. The racks containing the vials were randomly translated and rotated within the incubators on a daily basis to avoid any potential edge effect. Once emerged and dried out, the flies were killed and stored in 70% ethanol. The red-eyed individuals were then used. Both right and left wings were removed and mounted on slides in a 50% glycerol, 50% lactic acid medium. Fourteen to 30 males per genotype per temperature were used, leading to a sample size of 774 individuals (1548 wings) (Table 1).

Table 1. Mutations used in this study: abbreviations, alleles, signaling pathways in which the genes are involved and sample sizes.

Gene (abbreviation)	Allele	Genetic pathway	Sample size 18°C	Sample size 28°C
control	–	–	22	28
argos (aos)	Wll	Egfr	14	14
asteroid (ast)	kg07563	Egfr	28	25
cable (cbl)	kg03080	Egfr	18	18
CG3957/wmd	kg07581	unkown	22	25
decapentaplegic (dpp)	kg08191	TGF-β	30	25
downstream of receptor kinases (drk)	kg03077	Egfr	28	28
Mothers against Dpp (mad)	kg00581	TGF-β	27	18
mastermind (mam)	kg02641	Egfr	29	16
p38b	kg01337	TGF-β/Egfr	17	10
cAMP-dependent protein kinase 1 (Pka-C1)	BG02142	Hh	14	27
pointed (pnt)	kg04968	Egfr	16	29
rhomboid/rhomboid-2 (rho-stet)	kg07115	Egfr	20	17
rhomboid-6 (rho-6)	kg09603	Egfr	27	29
saxophone (sax)	kg07525	TGF-β	27	30
Src42A	kg02515	Egfr	30	28
Thickveins (tkv)	kg01923	TGF-β	25	13
		Total	394	380

Images of the wings were acquired with an Imasys U-eye digital camera mounted on a Leica DMRB microscope. Fifteen landmarks per wing were digitized using an ImageJ plugin written by Chris Klingenberg (pers. comm.; see Fig. 1).

MORPHOMETRIC ANALYSIS AND STATISTICAL TREATMENT

Superimposition

Generalized least squares Procrustes superimposition was used to extract shape variation from the landmark data (see for example Dryden and Mardia 1998). To avoid problems related to the loss of dimensions due to the superimposition, a principal component analysis (PCA) was applied to the Procrustes coordinates (i.e., the coordinates after superimposition) and the PC scores were used as shape variables in all subsequent shape analyses.

Log of the centroid size (i.e., the square root of the sum of the squared distances from each landmark to the centroid of the configuration) was used as a size variable.

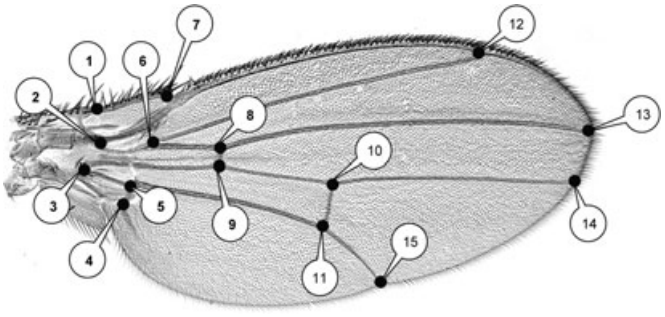

Figure 1. Position of the 15 landmarks on a wing.

Measurement error analysis

Measurement error (ME) is of critical importance when analyzing FA (e.g., Palmer 1994). To assess the significance of FA relative to ME, left and right wings of 30 individual flies were digitized twice. We then applied a conventional analysis of variance (ANOVA) to centroid size for size and a Procrustes ANOVA for shape, using individual, side, and their interaction as effects. The details of this procedure can be found in Palmer and Strobeck (1986, 2003), Palmer (1994), and Klingenberg and McIntyre (1998). In addition to the estimation of ME, this ANOVA allows testing for the significance of the individual effect and for the occurrence of DA.

Effects on mean size and shape

The effects of mutations, temperature, and their interaction on mean size and shape were investigated using ANOVAs on centroid size and multivariate analyses of covariance (MANCOVAs) on the shape variables (NB: the matrices can be inverted because they are computed from full rank matrices of PC scores). The following model was used:

$$W_{ijk} = \mu + G_i + T_j + G \times T_{ij} + \varepsilon_{ijk},$$

where W is the wing parameter, G is genotype, and T is temperature, all fixed effects. For shape, centroid size was added to the model as a covariate.

Effects on the levels of size and shape variation

The amount of individual variation and of FA for size and shape were measured for each genotype at both temperatures using

regular ANOVAs for size and Procrustes ANOVAs for shape, considering individual, side, and the interaction individual × side as effects. The MS related to the individual effect was used as an estimator of individual variation, and the MS related to the interaction (individual × side) was used as an estimator of FA. Size MS and Procrustes MS were then compared among temperatures and genotypes using standard *F*-tests.

As a first test of the relationship between canalization and developmental stability, we computed the correlation between individual variation and FA across genotypes at each temperature.

The effect of temperature on genetic variation for wing size and shape was assessed in measuring variation among genotypes. For both temperatures, this was computed as the MS in an ANOVA on size with genotype as a single effect. The corresponding Procrustes MS was used to measure shape variation across genotypes.

Effects on the patterns of shape variation

Do mutational and temperature-related shape variations involve similar changes in landmarks position? To investigate the qualitative effects of temperature and of insertions upon the directions (i.e., the patterns) of shape variation, we used the following approach. For each genotype at each temperature three matrices were computed, one corresponding to the differences among individuals, one to FA, and one to DA. These matrices are multivariate analogs of mean squares and depict the variation and covariation among landmarks associated to the different components of shape variation (mean squares and cross-products matrices [MSCP matrices]). Additionally, a matrix depicting the average effect of the temperature was computed for each genotype. To get a general picture of the relationship between these different components of variation, we used a principal coordinates analysis (PCO; also known as metric multidimensional scaling; Mardia et al. 1979; applications to similar datasets can be found in Debat et al. 2006, 2008). A distance measure for each pair of matrices was defined as one minus the squared correlation between the two matrices and used as input for metric multidimensional scaling. The diagonal of the compared matrices was not included to avoid any scaling effect and to focus on the covariation only. The resulting principal coordinates are axes that successively account for the maximum amount of the information contained in the corresponding distance matrix. Such an ordination allows one to visualize the relationship among matrices. Simply put, the closer two matrices are in the PCO plan, the more they are correlated, and the more similar the patterns of landmark variation. Although a purely descriptive ordination of the matrices, PCO was preferred over classical matrix comparison methods. The high number of matrices in our study together with their high dimensionality indeed precluded the use of more sophisticated methods such as CPCA (common principal component analysis, e.g., Klingenberg et al. 1996), which would have required a prohibitive amount of computer time. The chosen approach nevertheless provides a very appealing and intuitive way of assessing the relationship among multiple matrices at once. We applied this procedure at different levels, following a hierarchical strategy.

We first applied a PCO simultaneously to the matrices computed for each of the 17 genotypes, corresponding to the mean temperature effect, to the individual variation, DA, and FA at both 18°C and 28°C. This allowed us to capture in a single step the overall similarity of the effect of plasticity, of microenvironmental variation and of developmental noise (i.e., a total of 119 matrices at once). To more accurately depict the relationship between individual variation and FA, we ran a PCO on the corresponding matrices alone. Finally, PCOs applied separately to the individual variation and FA matrices allowed us to investigate the effects of temperature and of mutations onto these components of variation.

In all cases, we checked whether the effects of the mutations clustered according to the signaling pathways involving the corresponding genes. It has been suggested that Procrustes superimposition might alter the structure of covariance matrices due to the spread of variation across landmarks related to the least squares criterion (e.g., Walker 2000). This effect is nevertheless believed to be of limited importance when shape variation is low (Dryden and Mardia 1998: 287; Klingenberg and Monteiro 2005), which is clearly the case for *Drosophila* wings (e.g., Houle et al. 2003). Moreover, any covariance matrix computed from the superimposed configurations should be equally affected—if at all. There is thus no reason to suspect that the superimposition procedure might affect the relationship among covariance matrices.

Each time the analyses involved multiple comparisons, the *P*-values were adjusted using the Holm procedure (Holm 1999; Benjamini and Yekutieli 2001).

All morphometric and statistical analyses were conducted using the R-morph package (M. Baylac, pers. comm.) running on R version 2.6.2 (R Development Core Team 2008).

Results

MEASUREMENT ERROR

The results are reported in Table 2. For both size and shape, measurement error was found to be of smaller amplitude than true FA suggesting that error does not bias our estimation of FA (the interaction MS is respectively about 100 times higher than ME MS for shape and 200 times for size). No DA was detected for size or shape (the side effect was not statistically significant; see Pélabon and Hansen [2008] for a discussion on DA in insect wings). Antisymmetry (AS) was not detected: for size, the distributions of the right minus left values did not significantly depart from normality (a bimodal distribution is expected when AS occurs); for shape we

Table 2. Measurement error (ME). Size ME was assessed through a two-way mixed model ANOVA on centroid size with individual as a random effect and side as a fixed effect, and their interaction. Shape ME was assessed through the equivalent Procrustes ANOVA (see text). Shape MS were multiplied by 10^7 and size MS by 10^5.

Data	Sources of variation	MS	df	*F*	*P*-value
Shape	Individual	161,71	754	1,99	<0,0001
	Side	81,63	26	1	0,46
	Side × individual	81,31	754	98,67	<0,0001
	Residual	0,82	1560		
Size	Individual	1103	29	14,14	<0,0001
	Side	26	1	0,33	0,57
	Side × individual	78	29	205,8	<0,0001
	Residual	0,38	60		

computed the vectors of right–left difference in the shape space and no clustering of the R–L vectors was found.

EFFECTS OF THE MUTATIONS AND TEMPERATURE ON MEAN SIZE AND SHAPE

Both temperature and mutations were found to affect wing size and shape. Wing size is systematically smaller at 28°C. The effect of temperature on shape is more difficult to depict. From the PCA applied to the Procrustes coordinates, individuals clearly cluster on PC1 according to temperature (Fig. 2). Reconstructing the extreme wing configurations on this axis allows for visualization of the related shape change (Fig. 2). When temperature increases, the wing contracts along the anteroposterior axis, producing a somewhat thinner, elongated shape. Simultaneously, both cross-veins shift, from distal to more proximal positions. Remarkably, the posterior cross-vein does not only translate, but also slightly rotates, meaning that landmarks 10 and 11 do not move in the same direction. Statistical significance of these effects is shown when tested simultaneously on all mutants: both temperature and genotype effects as well as their interaction were found to be significant in the ANOVA on centroid size and in the MANCOVA on the PC scores (Table 3). This was confirmed in individual ANOVAs and MANCOVAs conducted on each genotype separately: for shape, all mutations had a significant effect and were found to interact with temperature (results not shown). For size, the effects of the insertions in *asteroid* (*ast*) and *Pka-C1* (*pka*) were not significant but their interaction with temperature was. In contrast, insertions in *cable* (*cbl*), *decapentaplegic* (*Dpp*), and *mastermind* (*mam*), while exhibiting a significant effect on size, did not significantly interact with temperature.

Altogether these results suggest that the heterozygous insertional mutations as well as the temperature significantly alter the size and shape of the wing.

Remarkably, the effects of the mutations are different at 18°C and 28°C for both size and shape (the interaction of temperature and mutations is statistically significant [Table 3]). Symmetrically, it means that the effect of temperature is different across mutants.

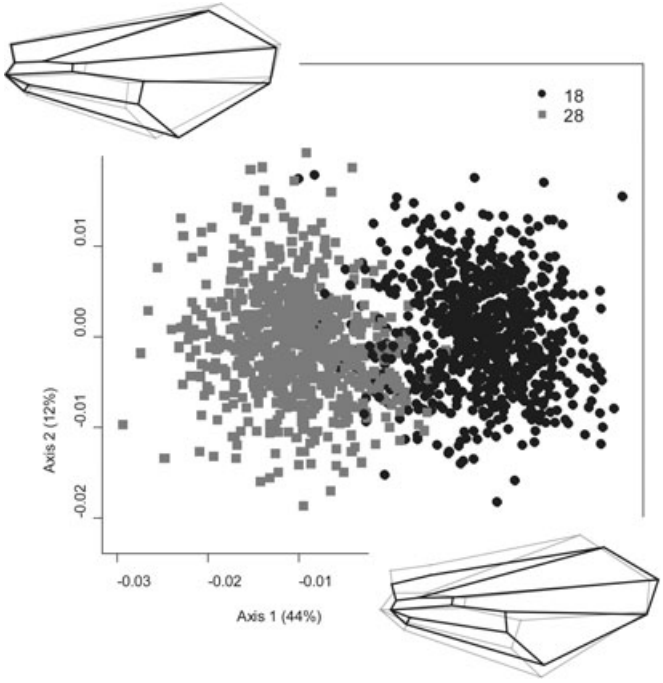

Figure 2. PCA on the whole dataset. Gray symbols: 28°C Black symbols : 18°C. The shape changes associated with the two PCs are visualized as configurations corresponding to extreme positions on the PCs. Gray, configurations for negative PC scores; black, configurations for positive scores. Shape changes correspond to an arbitrary value of 2.5 standard deviations.

Size reaction norms are shown in Figure 3. Noticeably, while the Oregon-R control flies reared at 28°C have a mean size lying in the middle of the range of genotypic effects, this is not observed at 18°C, where the mean size for all mutants is smaller than the control, with most of the differences being significant. Most reaction norms are roughly parallel to the wild-type reaction norm, illustrating the global common effect of temperature on size. However, some lines do cross each other, as expected from the significant interaction (temperature × genotypes) in the ANOVA, suggesting genetic differences in phenotypic plasticity across the set of mutations analyzed.

The results of a discriminant analysis including genotype and temperature as clustering variables are shown on Figure 4. The dataset is strongly structured by the temperature along the first canonical axis. Mutant genotypes exhibit strong effects on wing shape. Shape reaction norms can be represented as lines linking the position of each genotypic mean at 18°C and 28°C. Although most genotypes exhibit reaction norms roughly parallel to the control one (as shown in Fig. 4 for *drk* and *rho-6*), some reaction norms are clearly different (see *cg* or *pka* in the figure).

Table 3. Effects of the temperature and of the mutations on the mean size and shape. An ANOVA with temperature, genotype and the interaction (temperature×genotype) was applied to centroid size; the same effects were used in a MANCOVA on the scores of a PCA for shape including centroid size as a covariate.

	Effect	df	SS	MS	*F*	*P* value
Size	Temperature	1	9,5	9,5	24523,63	<0.0001
	Genotype	16	0,24	0,015	38,95	<0.0001
	Temperature×genotype	16	0,08	0,005	13,23	<0.0001
	Residuals	1514	0,58	0,0004		

	Effect	df	Pillai	F	Df num	Df den	Pr(>F)
Shape	Temperature	1	0,93	822,66	26	1488	<0.0001
	Genotype	16	4,45	22,28	416	24048	<0.0001
	Size	1	0,33	28,56	26	1488	<0.0001
	Temperature×genotype	16	2,02	8,36	416	24048	<0.0001
	Residuals	1513					

This illustrates the significant interaction genotype × temperature in the MANCOVA.

Concerning genetic variation, temperature had no detectable effect on size or shape as the MS related to the genotype effect were not different among temperatures (Size $F_{16,16} = 1.181$ $P = 0.37$; Procrustes $F_{416,416} = 1.022$, $P = 0.408$)

EFFECTS OF MUTATIONS AND TEMPERATURE ON SIZE AND SHAPE VARIATION

Concerning size (Fig. 5A, B), for most genotypes, the extent of individual variation and FA are greater at 18°C than at 28°C. At 18°C, all genotypes exhibit levels of individual variation higher than the control, nine of which remain significantly higher after a Holm procedure for multiple comparisons was conducted (Holm 1999; Benjamini and Yekutieli 2001). For FA, the mutants also tend to be more asymmetric than the controls (Fig. 5B left), but none of the 16 pairwise comparisons remains significant after adjustment. No trend is detected at 28°C for individual variation or FA: the effects on size variation strongly depend on the mutation (as for 18°C, no difference with control remains significant after adjustment).

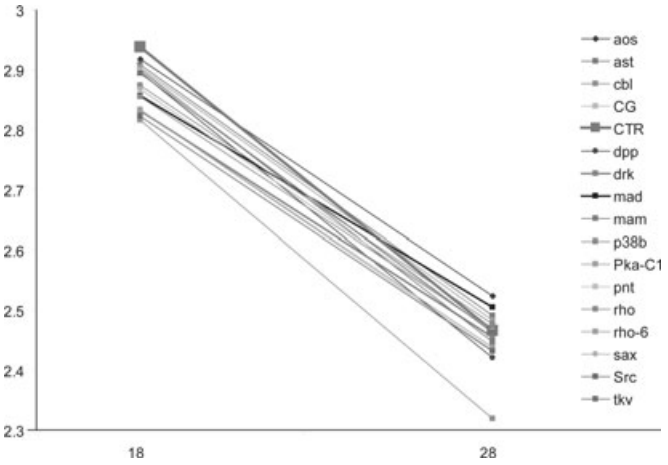

Figure 3. Wing size reaction norms for all genotypes. Size is measured as centroid size. Large gray square: control. The lowest line is P38.

In contrast with size, shape individual variation and FA (Fig. 5C, D) are not systematically higher at 18°C, even though a slight trend can be found for individual variation. The most obvious effect is that of the mutations on individual variation, which is in most cases higher in mutant genotypes relative to the control (Fig. 5C). The effect of mutations on FA is largely dependent on the mutation. Significant increase in FA relative to the control was recorded in only three genotypes at 18°C (*pnt*, *drk,* and *mam,* Fig. 5D left), and two genotypes at 28°C (*mad* and *mam,* Fig. 5D right).

Noticeably, as already found for size at 28°C (Fig. 5 right), *mam* mutants present the highest levels of shape FA for both temperatures.

For size as for shape, the most variable genotypes at one temperature were not found to be the most variable at the other: the levels of individual variation across genotypes at 18°C were not correlated with those at 28°C ($r_{\text{size}} = -0.05$, $P = 0.69$; $r_{\text{shape}} = 0.07$ $P = 0.72$), and neither were the FA levels ($r_{\text{size}} = 0.1$, $P = 0.51$; $r_{\text{shape}} = 0.58$, $P = 0.06$).

Considering the relationship between individual variation and FA, for both temperatures, the most variable genotypes were also the most asymmetric for size, as shown by the correlation between levels of individual variation and of FA at both temperatures ($r_{18°C} = 0.63$, $P = 0.028$; $r_{28°C} = 0.58$, $P = 0.045$). This was not the case for shape, where individual variation and FA were not correlated ($r_{18°C} = 0.48$, $P = 0.15$; $r_{28°C} = 0.35$, $P = 0.33$).

Interestingly, shape DA was detected for most genotypes (and noticeably for the control at both temperatures) (a significant side

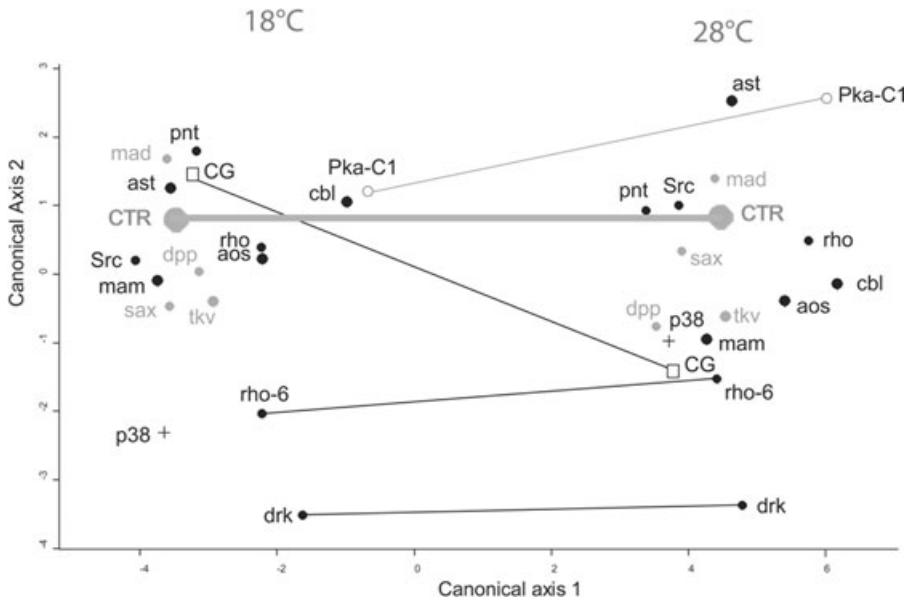

Figure 4. Shape reaction norms: discriminant analysis with temperature and genotype as factors. The symbols represent the position of the genotype means. Large gray symbols, control means; gray symbols, genes from the TGF-β signaling pathway; black symbols, genes from the Egfr signaling pathway; open circle, genes from the hedgehog pathway; cross, genes involved in both Egfr and TGF-β pathways; open square, genes with no known role in a signaling pathway; connecting lines represent the direction of shape change due to temperature within a genotype, i.e., shape reaction norms.

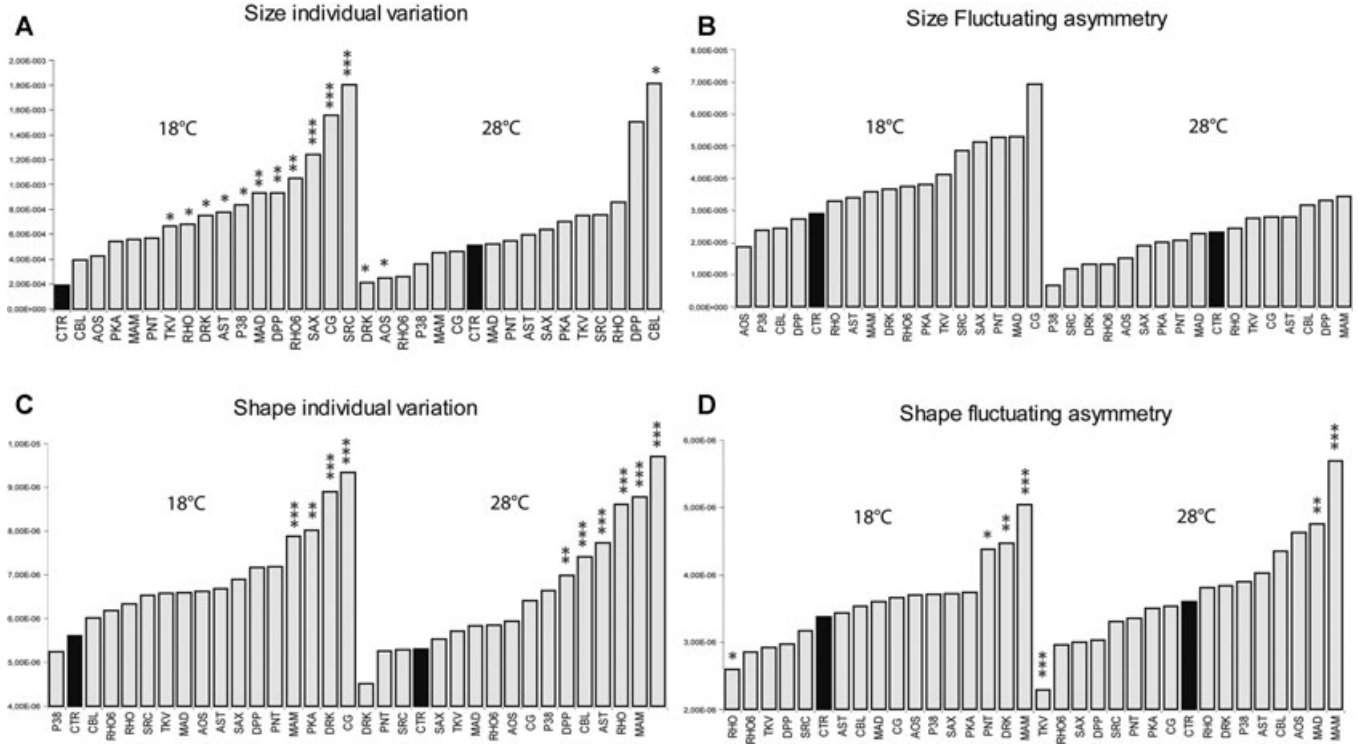

Figure 5. Levels of individual variation and FA for size (A and B) and shape (C and D). Each graphic shows the values corresponding to18°C (left) and 28°C (right). Within each temperature, the values have been ranked from lowest (left) to highest (right). Black bars, control; gray bars, mutants. Stars indicate the genotypes whose variation remains significantly higher than that of the corresponding control after adjustment for multiple comparisons. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

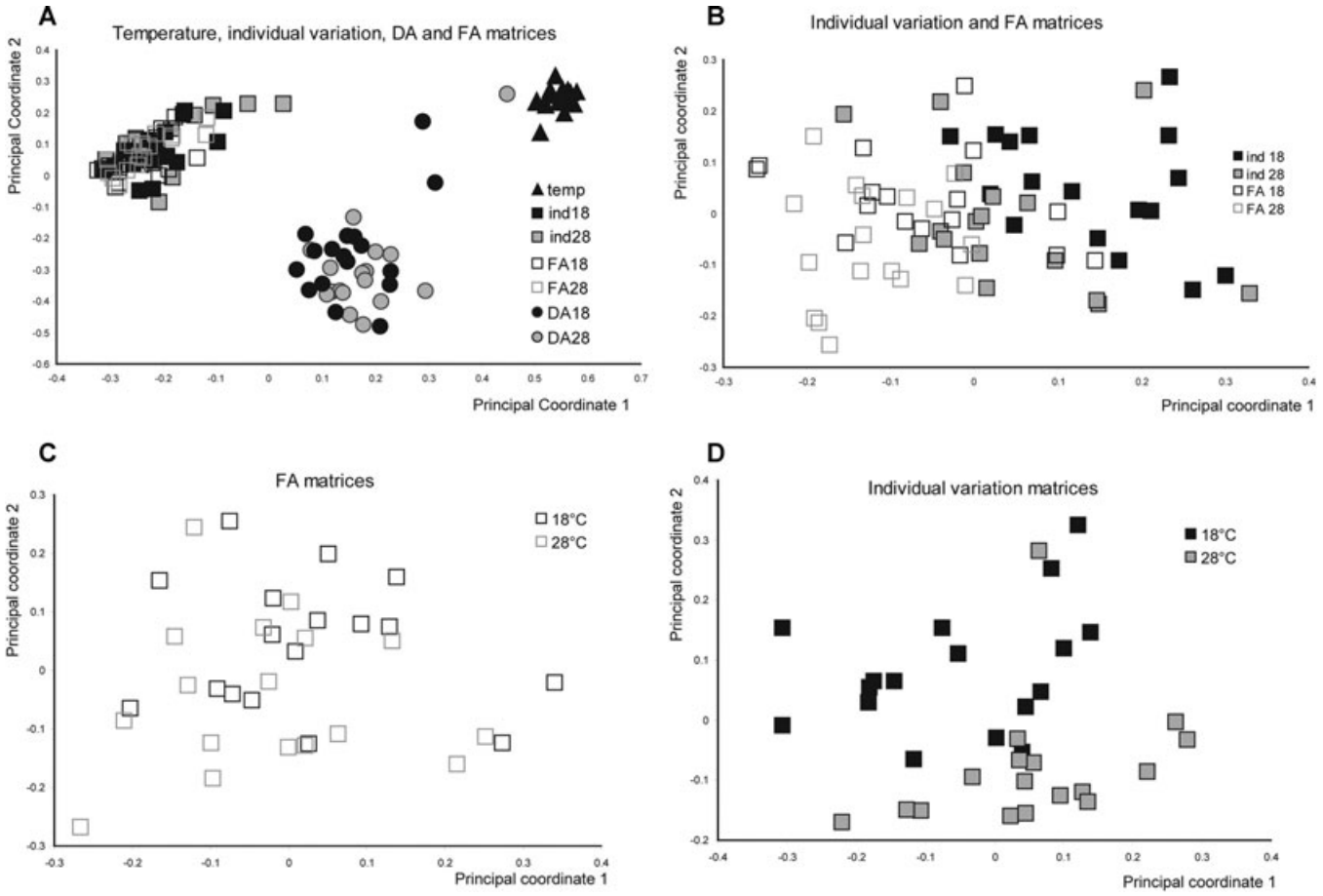

Figure 6. Principal coordinates analyses (PCOs) of the shape matrices. Each symbol represents a single matrix (i.e., a genotype at a given temperature). Black triangles, effect temperature; black squares, individual variation at 18°C, gray squares, individual variation at 28°C; open black squares, FA at 18°C; open gray squares, FA at 28°C; black circles, DA at 18°C; gray circles, DA at 28°C.

effect was found in the Procrustes ANOVAs). The amount of DA varied across genotypes and between temperatures although no trend could be detected. DA was not detected for size.

The temperature did not affect genetic variation for size or shape, as the MS related to genotype effect in regular and Procrustes ANOVAs did not significantly differ across temperatures ($F_{\text{size}(16,16)} = 1.118\ P = 0.37$; $F_{\text{shape}(391,391)} = 1.022\ P = 0.41$).

PATTERNS OF SHAPE VARIATION

Results of the PCOs are shown in Figure 6. From the first ordination applied simultaneously to the different types of variation matrices (Fig. 6A), it appears that the matrices of temperature effects tightly cluster together, and are far from the other matrices. Directional asymmetry matrices of both temperatures cluster together, clearly apart from the other types of matrices. This is true for all genotypes, except *p38* at 28°C (and to a lesser extent *ast* and *pnt* at 18°C), which surprisingly falls near the temperature effect matrices. Individual variation and FA matrices of both temperatures cluster together in this general ordination. Results of the PCO applied to the individual variation and FA matrices are shown in Figure 6B–D. Interestingly, the matrices of individual variation are relatively distinct from the FA matrices for both temperatures (Fig. 6B). There is indeed a shift in the position of the two types of matrices along the first PCO, irrespective of genotype and temperature suggesting a systematic difference between these matrices. When focusing on FA matrices only (Fig. 6C), no evidence for any structuration is found. Temperature, therefore, does not appear to alter the structure of FA matrices. In a striking contrast, in the Figure 6D, individual matrices clearly cluster according to the temperature.

Finally, no clustering of mutations effects relative to the signaling pathways was detected in this analysis, consistent with previous observations (Dworkin and Gibson 2006).

Discussion

SIZE AND SHAPE

Dworkin and Gibson (2006) using the same lines at 25°, found little evidence for an effect of the mutations on size and therefore suggested that the heterozygous insertional mutations largely

altered wing shape, but not size. Our results show that this is not always the case. Whereas shape is found to be clearly affected by the mutations at both temperatures, the effects on size are conditional on rearing environment: at 28°C no significant difference from the control is found for most genotypes. At 18°C however, the mutations consistently induce a decrease in wing size. The current study used an additional five landmarks in the posterior-proximal region of the wing, as compared to the original study. It is conceivable that the size effects could be spatially limited, remaining undetected in Dworkin and Gibson's study. However, our results were not affected when conducting the same analysis after the additional landmarks had been removed, providing no support for this hypothesis. Alternatively, because only a subset of the original mutations was used in this study, the discrepancy could have resulted from a difference in the statistical power related to the number of comparisons made (16 in the present study vs. 50 in the previous one). To test this, we adjusted the *P*-values according to 50 instead of 16 comparisons. The resulting *P*-values remained significant in most cases suggesting that the difference between the studies is not related to a statistical power issue. The most likely explanation for our results is that the size effects of the mutations are dependent on rearing temperature.

The difference between the effects on size and shape suggests a relative independence between these two components of form. This is intriguing given their tight mechanical relationship: wing shape, as assessed in this study, depends on the relative positions of the veins, which, in turn, depends on the relative size and shape of the different intervein regions. It has been suggested that size variation might be more sensitive than shape to small variations in the environmental context, especially in the available resources (Breuker et al. 2006). Many studies have pointed at similar contrast between size and shape variation (e.g., Gilchrist and Partridge 2001; Debat et al. 2003). The shape of *Drosophila* wing is known for its evolutionary conservation across species from the genus (and even beyond; e.g., Houle et al. (2003)). Such stability might result either from developmental or structural constraints, a lack of genetic variation, or strong stabilizing selection (or all of these). The latter hypothesis seems the most likely because strong response to artificial selection on wing shape have been repeatedly obtained (Weber 1990, 1992; Houle et al. 2003; Pélabon et al. 2006). This indeed demonstrates that genetic variation occurs and that no absolute constraint prevents the wing shape to vary. The nature of the specific selective pressure is however unknown, a part from some obvious guess on the importance of wing shape for flight or male courtship song, which, however, remains to be rigorously tested (but see Routtu et al. (2007) and Frazier et al. (2008) for recent contributions). Additionally, it is difficult to explain why such selective pressure would act more strongly on shape than on size. More research is needed to understand the relative role of wing size and shape in its diverse functions, as well as on the developmental processes linking these two aspects of form (e.g., Resino et al. 2002).

THE GENETICS OF PLASTICITY FOR WING SIZE AND SHAPE: ALLELIC SENSITIVITY?

The temperature-dependent effect of the mutations points to the general question of the genetic bases of plasticity. Although it has been clearly shown that plasticity evolves and thus has a genetic basis (e.g., Windig et al. 2004, for a review), it remains nevertheless unclear whether the genes contributing to set up a trait's value in given environmental conditions are also responsible for the trait's plasticity. This question was at the core of the controversy on the genetic bases of plasticity (Via et al. 1995). Schematically, the argument opposed the view that the genes involved in a trait morphogenesis are themselves sensitive to environmental conditions—the pleiotropic hypothesis or allelic sensitivity, or to the existence of specific mechanisms devoted at translating environmental inputs into phenotypic outcomes—the epistatic hypothesis or the "genes for plasticity" hypothesis (see Scheiner 1993 and Via et al. 1995 for reviews). Pigliucci (2005) suggested that this controversy is now obsolete, considering that "both pleiotropic and epistatic effects are characteristic of any plastic response that has been extensively investigated to date." However, it is likely that the question remains open precisely because the cases in which the genetic bases of plasticity are well documented are few.

Our results show that heterozygous insertional mutations affecting genes involved in wing development can modify plasticity to temperature for the size and shape of the *Drosophila* wing. For most genotypes, the size and shape reaction norms are indeed modified when compared to the wild type (Figs. 3 and 4), which is congruent with the pleiotropic or allelic sensitivity hypothesis.

However, this does not necessarily mean that the genes used in this study are actually responsible for the plastic response observed in the wild-type flies. It is indeed conceivable that a change in the regulation of these or other genes would induce similar effects on wing plasticity. Our data therefore do not allow us to clearly favor one hypothesis over the other. However, because most heterozygous mutations had an effect on plasticity (either on size or shape), it remains possible that polymorphisms at these loci could contribute to the variation for wing plasticity found in natural populations.

EFFECTS ON WING CANALIZATION AND DEVELOPMENTAL STABILITY

Because the lines are nearly isogenic, variation among flies within a line mostly reflects microenvironmental differences. In addition to the effect of the mutations on plasticity, our results therefore suggest that the mutants' sensitivity to microenvironmental variation is also increased relative to the wild type. In other words, the

mutations seem to impair environmental canalization. This is in agreement with the general belief that mutants are phenotypically less robust than the wild type (e.g., Waddington 1942, 1953), but contrasts with the findings of Dworkin and Gibson (2006) who detected minimal effects of the insertions on the within-line variation when measured at 25°C. Again, as for plasticity, the clearest effects are found at 18°C where most mutant genotypes exhibit for both size and shape higher levels of individual variation relative to the control (Fig. 5A,C). The temperatures used in the current study are likely stressful to flies (e.g., Pétavy et al. 2001; Debat et al. 2003). It is thus possible that they would act synergistically with the mutations, increasing the observed phenotypic variation. This hypothesis however is not free of complications because 18°C is generally considered less stressful than 28°C (Pétavy et al. 2001). Clearly, more work is needed on the effects of temperature on wing phenotypic and genetic variability.

Most of the recent discussion about canalization has concerned its genetic component (e.g., Visser et al. 2003), because it theoretically allows for the build up—and thus the occasional release—of cryptic genetic variation, potentially altering the pace of morphological evolution (Rutherford and Lindquist 1998; Gibson and Dworkin 2004). The experimental design used in this study could not allow us to assess the effects of mutations on genetic variation. In turn, we could estimate the effect of temperature on genetic variation, by comparing variation across genotypes under both environmental conditions. Because no difference was detected, genetic canalization is apparently not affected by temperature. This result therefore suggests that it is possible to change environmental canalization with no effect on genetic canalization, in contradiction with the hypothesis of genetic canalization evolving as a byproduct of environmental canalization (e.g., Meiklejohn and Hartl 2002; but see Dworkin 2005 for a discussion). This result should nevertheless be considered with caution because the variation among 16 isogenic lines does not provide a very reliable estimator of genetic variation.

The clearest effect on within-line variation is that of the temperature. Concerning size, for most genotypes both individual variation and FA increase at 18°C (Fig. 5A,B). This suggests that at 18°C size environmental canalization and developmental stability are less efficient than at 28°C. As for the effect of the mutations, shape variation seems less affected than size (Fig. 5C,D). This effect of temperature on individual variation and FA illustrates that environmental as well as genetic factors can alter canalization and developmental stability. Additionally, it suggests that the two components of developmental homeostasis can react similarly to temperature. This is consistent with the significant correlation between FA and individual variation across genotypes for size—but not for shape—at both temperatures. This points at the relationship between the processes buffering phenotypic variation: does robustness against microenvironmental variation involve anything else than the robustness against developmental noise?

THE LINK BETWEEN CANALIZATION AND DEVELOPMENTAL STABILITY

The relationship between canalization and developmental stability has generated some debate in the past decade. Some authors have suggested that there is no need for more than one mechanism to account for developmental homeostasis (e.g., Clarke 1998; Klingenberg and McIntyre 1998; Breuker et al. 2006), whereas others have proposed that canalization and developmental stability are at least partly different (Réale and Roff 2003; Santos et al. 2005; Debat et al. 2006, 2008; Rego et al. 2006) some authors having even suggested that the two processes could be independent (Debat et al. 2000). In these studies, the data typically consist in the levels and patterns of phenotypic variation among and within individuals, the relationship between canalization and developmental stability being measured as the correlation between the two types of variation.

Our observations from the current study provide different elements to this discussion. First, concerning size, individual variation and FA are similarly affected by mutations and by temperature. This is strengthened by the correlation of the levels of size individual variation and size FA across genotypes for both temperatures. For wing shape, in contrast, we observe that individual variation is systematically higher in mutants, whereas shape FA does not present such a consistent trend, and the correlation between shape FA and shape individual variation is not significant. These mixed results are consistent with the hypothesis that the relationship between canalization and developmental stability is trait specific, as previously suggested in the literature (e.g., Hoffmann and Woods 2001).

The PCOs (Fig. 6) provide additional cues to this question. The most general ordination including the plasticity effect and DA in addition to the individual variation and FA (Fig. 6A), shows that the characters involved in plasticity are different from those varying among individual and those involved in FA—or DA. This analysis showing the individual variation and FA matrices clustering together indicates that the related patterns of shape variation are very close. However, when specifically focusing on these two types of matrices, some differences appear: first the individual and FA matrices seem to differ, irrespective to temperature (Fig. 6B), suggesting that although close, the patterns of related variation are not identical. Finally, the systematic effect of temperature on individual variation but not FA matrices (Fig. 6C,D) indicates that the processes involved in the buffering of intra and inter individual variation are not completely similar. Such a difference needs to be addressed in terms of developmental processes and molecular mechanisms. Unfortunately, very little is known about the genetic control of symmetry and asymmetry in bilaterians in

spite of recent progress (e.g., Speder et al. 2006; see Levin and Palmer 2007 for a review), and this incomplete knowledge sets limits to the interpretation of our results. This is particularly frustrating given the contrasted conclusions across studies, even when investigating similar traits using similar methodologies (e.g., the *Drosophila* wing shape; Santos et al. 2005; Breuker et al. 2006; Debat et al. 2006, 2008). Additional experimental investigations of the developmental bases of morphological variation are therefore needed.

IMPACT ON EVOLUTIONARY POTENTIAL

The systematic clustering of individual variation matrices relative to temperature means that the phenotypic variation expressed at one temperature is relatively stable across genotypes, but consistently different from phenotypic variation expressed at another temperature. In addition to directly changing the direction of selection (a new environment is likely to generate new selective pressures), the temperature therefore simultaneously alters the main direction of available phenotypic variation.

Bearing in mind that the component of phenotypic variation considered here is mostly nongenetic (i.e., the individual variation matrices are computed within each of the isogenic lines), one might wonder about the importance for selection of such an effect. However, some recent work has advocated the primacy of phenotypic change over genetic variation in evolution via phenotypic accommodation (see West Eberhard 2003; Palmer 2004; Badyaev 2005; Braendle and Flatt 2006, for general discussions). This suggests that temperature, in systematically altering the structure of individual phenotypic variation, might alter as well the population's potential for further evolution.

This analysis should be extended to include all of the available genotypes: only a fraction of the genes initially used by Dworkin and Gibson (2006) were used in the current study. Nevertheless, the results obtained, even on the limited basis of a controlled laboratory experiment, are promising. They point at the actual complexity of the real world in which genes are not the only parameters involved in shaping phenotypes. Disentangling the respective effects of mutations and environment is obviously challenging but nevertheless possible given an appropriate model and methodology are used. Clearly, environmental manipulations should systematically be integrated into classical genetic designs to gain a proper understanding of organism's variational—and thus evolutionary—properties.

ACKNOWLEDGMENTS

We are grateful to M. Wayne and two anonymous reviewers for their thoughtful comments that greatly improved the manuscript. Thanks to A. Evin and M. Baylac for discussions on morphometrics and statistic treatments during the preparation of this manuscript. VD was supported by a Marie Curie European reintegration Grant.

LITERATURE CITED

Allen, C. E., P. Beldade, B. J. Zwaan, and P. M. Brakefield. 2008. Differences in the selection response of serially repeated color pattern characters: standing variation, development, and evolution. BMC Evol. Biol. 8:94.

Badyaev, A. V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proc. R. Soc. B 272:877–886.

Barrett, R. D., S. M. Rogers, and D. Schluter. 2008. Natural selection on a major armor gene in threespine stickleback. Science. 322:255–257.

Beldade, P., and P. M. Brakefield. 2002. The genetics and evo-devo of butterfly wing patterns. Nature Rev. Genet. 3:442–452.

Benjamini, Y., and D. Yekutieli. 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29:1165–1188.

Braendle, C., and T. Flatt. 2006. A role for genetic accommodation in evolution? BioEssays 28:868–873.

Breuker, C. J., J. S. Patterson, and C. P. Klingenberg. 2006. A single basis for developmental buffering of *Drosophila* wing shape. PloS ONE 1:e7.

Brunetti, C. R., J. E. Selegue, A. Monteiro, V. French, P. M. Brakefield, and S. B. Carroll. 2001. The generation and diversification of butterfly eyespot color patterns. Current Biology 11:1578–1585.

Callahan, H. S., and M. Pigliucci. 2002. Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis thaliana*. Ecology 83:1965–1980.

Clarke, G. M. 1998. The genetic basis of developmental stability, V. Inter- and intra-individual character variation. Heredity 80:562–567.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal Jr., M. Dickson, J. Grimwood, J. Schmutz, R. M. Myers, D. Schluter, and D. M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. Science. 307:1928–1933.

Cresko, W. A., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. A. Bell, C. B. Kimmel, and J. H. Postlethwait. 2004. Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. Proc. Natl. Acad. Sci. USA. 2101:6050–6055.

David, J. R., B. Moreteau, J. P. Gauthier, G. Petavy, J. Stockel, and A. G. Imasheva. 1994. Reaction norms of size characters in relation to growth temperature in Drosophila melanogaster: an isofemale lines analysis. Genet. Sel. Evol. 26:229–251.

David, J. R., H. Legout, and B. Moreteau. 2006. Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply. J. Genet. 85:9–23.

Debat, V., and P. David. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol. Evol. 16:555–561.

Debat, V., P. Alibert, P. David, E. Paradis, and J.-C. Auffray. 2000. Independence between developmental stability and canalization in the skull of the house mouse. Proc. R. Soc. Lond. B 267:423–430.

Debat, V., M. Begin, H. Legout, and J. R. David. 2003. Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature. Evolution 57:2773–2784.

Debat, V., C. C. Milton, S. L. Rutherford, C. P. Klingenberg, and A. A. Hoffmann. 2006. Hsp90 and the quantitative variation of the shape of *Drosophila* wing. Evolution 60:2529–2538.

Debat V., R. Cornette, A. B. Korol, E. Nevo, D. Soulet, and J. R. David. 2008. Multidimensional Analysis of Drosophila wing variation in Evolution Canyon. J. Genet. 87:407–419.

De Celis, J. F. 2003. Pattern formation in the *Drosophila* wing: the development of the veins. Bioessays 25:443–451.

Dryden, I. L., and K. V. Mardia. 1998. Statistical shape analysis. Wiley, Chichester.

Dworkin, I. 2005. A study of canalization and developmental stability in the sternopleural bristle system of *Drosophila melanogaster*. Evolution 59:1500–1509.

Dworkin, I., and G. Gibson. 2006. Epidermal growth factor receptor and transforming growth factor-β signaling contributes to variation for wing shape in *Drosophila melanogaster*. Genetics 173:1417–1431.

Flatt, T. 2005. The evolutionary genetics of canalization. Q. Rev. Biol. 80:287–316.

Frazier, M. R., J. Harrison, S. D. Kirkton, and S. P. Roberts. 2008. Cold rearing improves cold-flight performance in *Drosophila* via changes in wing morphology. J. Exp. Biol. 211:2116–2122.

Gibert, J.-M., F. Peronnet, and C. Schlötterer. 2007. Phenotypic plasticity in *Drosophila* pigmentation caused by temperature sensitivity of a chromatin regulator network. Plos Genetics 3:e30.

Gibson, G., and I. Dworkin. 2004. Uncovering cryptic genetic variation. Nat. Rev. Genet. 5:681–690.

Gilchrist, A. S., and L. Partridge. 2001. The contrasting genetic architecture of wing size and shape in *Drosophila melanogaster*. *Heredity* 86:144–152.

Gilchrist, G. W., R. B. Huey, J. Balanyà, M. Pascual, and L. Serra. 2004. A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. Evolution 58:768–780.

Gompel, N., B. Prud'homme, P. J. Wittkopp, V. A. Kassner, and S. B. Carroll. 2005. Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in *Drosophila*. Nature 433:481–487.

Hallgrimsson, B., and B. K. Hall. 2005. Variation. Academic Press, SanDiego.

Hoffmann, A. A., and R. E. Woods. 2001. Trait variability and stress: canalization, developmental stability and the need for a broad approach. Ecol. Lett. 4:97–101.

Holm, S. 1999. Multiple confidence sets based on stagewise test. J. Am. Statist. Soc., 94:489–495.

Houle D., J. Mezey, P. Galpern, and A. Carter. 2003. Automated measurement of Drosophila wings. BMC Evol. Biol. 3:25.

Imasheva, A. G., B. Moreteau, and J. R. David. 2000. Growth temperature and genetic variability of wing dimensions in Drosophila: opposite trends in two sibling species. Genet. Res. Camb. 76:237–247.

Jeong, S., M. Rebeiz, P. Andolfatto, T. Werner, J. True, and S. B. Carroll. 2008. The evolution of gene regulation underlies a morphological difference between two *Drosophila* sister species. Cell 132:783–793.

Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52:1363–1375.

Klingenberg, C. P., and L. R. Monteiro. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst. Biol. 54:678–688.

Klingenberg, C. P., B. E. Neuenschwander, and B. D. Flury. 1996. Ontogeny and individual variation: analysis of patterned covariance matrices with common principal components. Syst. Biol. 45:135–150.

Levin, M., and A. R. Palmer. 2007. Left-right patterning from the inside out: widespread evidence for intracellular control. BioEssays 29, 271–287.

Marchinko K. B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution. 61:1084–1090.

Mardia, K. V., J. T. Kent, and J. M. Bibby. 1979. Multivariate analysis. Academic Press, London.

McGuire, R., and A. A. Agrawal. 2005. Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*. Funct. Ecol. 19:1025–1031.

McKinnon, J. S., and H. D. Rundle. 2002. Speciation in nature: the threespine stickleback model systems. Trends Ecol. Evol. 17:480–488.

Meiklejohn, C. D., and D. L. Hartl. 2002. A single mode of canalization. Trends Ecol. Evol. 17:468–473.

Milton, C. C., B. Huynh, P. Batterham, S. L. Rutherford, and A. A. Hoffmann. 2003. Quantitative trait symmetry independent of Hsp90 buffering: distinct modes of genetic canalization and developmental stability. Proc. Natl. Acad. Sci. USA 100:13396–13401.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer. Pp. 335–364 *in* T. Markow, ed. Developmental instability: its origins and evolutionary implications. Kluwer Academic Publishers, Dordrecht.

———. 2004. Symmetry breaking and the evolution of development. Science 306:828–833.

Palmer, A. R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst. 17:391–421.

———. 2003. Fluctuating asymmetry analyses revisited. Pp. 279–319 *in* M. Polak, ed. Developmental instability: causes and consequences. Oxford Univ. Press, Oxford, U.K.

Partridge, L., B. Barrie, K Fowler, and V. French. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48:1269–1276.

Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. Cole, P. F. Colosimo, C. A. Buerkle, D. Schluter, and D. M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901–905.

Pélabon C., and T. F. Hansen. 2008. On the adaptive accuracy of directional asymmetry in insect wing size. Evolution 62:2855–2867.

Pélabon, C., M. L. Carlson, T. F. Hansen, N. G. Yoccoz, and W. S. Armbruster. 2004. Consequences of inter-population crosses on developmental stability and canalization of floral traits in *Dalechampia scandens* (Euphorbiaceae). J. Evol. Biol. 17:19–32.

Pélabon, C., T. F. Hansen, A. J. R. Carter, and D. Houle. 2006. Response of fluctuating and directional asymmetry to selection on wing shape in *Drosophila melanogaster*. J. Evol. Biol. 19:764–776.

Pétavy, G., J. R. David, P. Gibert, and B. Moreteau. 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. J. Therm. Biol. 26:29–39.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20:481–486.

R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.

Réale, D., and D. A. Roff. 2003. Inbreeding, developmental stability, and canalization in the sand cricket *Gryllus firmus*. Evolution 57:597–605.

Rego, C., M. Matos, and M. Santos. 2006. Symmetry breaking in interspecific drosophila hybrids is not due to developmental noise. Evolution 60:746–761.

Resino, J., P. Salama-Cohen, and A. Garcia-Bellido. 2002. Determining the role of patterned cell proliferation in the shape and size of the drosophila wing. Proc. Natl. Acad. Sci. USA. 99:7502–7507.

Rohlf, F. J., and L. F. Marcus. 1993. A revolution in morphometrics. Trends Ecol. Evol. 8:129–132.

Routtu, J., D. Mazzi, K. Van Der Linde, P. Mirol, R. K. Butlin, and A. Hoikkala. 2007. The extent of variation in male song, wing and genital characters among allopatric *Drosophila montana* populations. J. Evol. Biol. 20:1591–1601.

Rutherford, S. L., and S. Lindquist. 1998. Hsp90 as a capacitor for morphological evolution. Nature 396:336–342.

Santos, M., P. Iriarte, and W. Cespedes. 2005. Genetics and geometry of canalization and developmental stability in *Drosophila subobscura*. BMC Evol. Biol. 5:7.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Ann. Rev. Ecol. Syst. 24:35–68.

Smith, H., and G. C. Whitelam. 1997. The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant Cell Environ. 20:840–844.

Speder, P., G. Adam, and S. Noselli. 2006. Type ID unconventional myosin controls left-right asymmetry in *Drosophila*. *Nature* 440:803–807.

Via, S., R. Gomulkiewicz, G. Dejong, S. M. Scheiner, C. D. Schlichting, and P. H. Vantienderen. 1995. Adaptive phenotypic plasticity—consensus and controversy. Trends Ecol. Evol. 10:212–217.

Visser, J. A. G. M., J. Hermisson, G. P. Wagner, L. A. Meyers, H. Bagheri-Chaichian, J. L. Blanchard, L. Chao, J. M. Cheverud, S. F. Elena, W. Fontana, et al. 2003. Perspective: evolution and detection of genetic robustness. Evolution 57:1959–1972.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. Nature 150, 563–565.

———. 1953. The genetic assimilation of an acquired character. Evolution 7:118–126.

Walker, J. A. 2000. Ability of geometric morphometric methods to estimate a known covariance matrix. Syst. Biol. 49:686–696.

Weber, K. E. 1990. Selection on wing allometry in *Drosophila melanogaster*. Genetics 126:975–989.

———. 1992. How small are the smallest selectable domains of form? Genetics 130:345–353.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford Univ. Press. Oxford.

Williams, T. M., J. E. Selegue, T. Werner, N. Gompel, A. Kopp, and S. B. Carroll. 2008. The regulation and evolution of a genetic switch controlling sexually dimorphic traits in Drosophila. Cell. 134:610–623.

Willmore, K. E., and B. Hallgrimsson. 2005. Within individual variation: developmental noise versus developmental stability. Pp. 191–218 *in* B. Hallgrimsson and B. K. Hall, eds. Variation: a central concept in biology. Elsevier, Burlington, MA.

Windig, J. J., C. G. F. De Kovel, and G. De Jong. 2004. Genetics and mechanics of plasticity. Pp. 31–49 *in* T. J. De Witt and S. M. Scheiner, eds. Phenotypic plasticity, functional and conceptual approaches. Oxford Univ. Press, Oxford.

Associate Editor: M. Wayne