



Response of fluctuating and directional asymmetry to selection on wing shape in *Drosophila melanogaster*

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Abstract

We tested whether directional selection on an index-based wing character in *Drosophila melanogaster* affected developmental stability and patterns of directional asymmetry. We selected for both an increase (up selection) and a decrease (down selection) of the index value on the left wing and compared patterns of fluctuating and directional asymmetry in the selection index and other wing traits across selection lines. Changes in fluctuating asymmetry across selection lines were predominantly small, but we observed a tendency for fluctuating asymmetry to decrease in the up-selected lines in both replicates. Because changes in fluctuating asymmetry depended on the direction of selection, and were not related to changes in trait size, these results fail to support existing hypotheses linking directional selection and developmental stability. Selection also produced a pattern of directional asymmetry that was similar in all selected lines whatever the direction of selection. This result may be interpreted as a release of genetic variance in directional asymmetry under selection.

Introduction

The degree of developmental precision varies dramatically across traits (Lajus *et al.*, 2003; Hansen *et al.*, 2006), but the reasons for this are poorly understood. Some have suggested that directional selection decreases the level of developmental precision or developmental stability (Soulé, 1967; Parson, 1992; Møller & Pomiankowski, 1993). This idea is rooted in Waddington's work on developmental canalization, in which he suggested that canalization increases with stabilizing selection (Waddington, 1957). Thus, while stabilizing selection may favour processes decreasing phenotypic variance (Wagner *et al.*, 1997; Rice, 1998; de Visser *et al.*, 2003; Hermisson *et al.*, 2003), directional selection may prevent the evolution of canalization and possibly favour mechanisms that increase phenotypic variation such as a

decrease in developmental stability (Rice, 1998; Kawecki, 2000; Hermisson & Wagner, 2004; Carter *et al.*, 2005).

Additional hypotheses linking directional selection with a decrease in developmental stability have been suggested. These include a genetic correlation between the expression of a trait and its sensitivity to developmental noise (Gavrilets & Hastings, 1994), occurrence of developmental homeostasis, i.e. trade-off between growth rate and regulatory processes during ontogeny (Calow, 1982; Arendt, 1997), or an indirect effect of directional selection on developmental stability due to the negative effect of homozygosity, resulting from selection, on developmental stability (Lerner, 1954; Soulé, 1967; Leamy, 1986). Interestingly, different predictions concerning the effect of selection in opposite directions follow from these hypotheses (Table 1).

Measuring developmental stability is intrinsically difficult because it consists of estimating the within-individual variance around an optimal value that is unknown in most cases. One exception, however, consists in measuring fluctuating asymmetry, small nondirectional departures from perfect bilateral symmetry (Van Valen, 1962). In bilateral characters, both sides

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Table 1 Hypotheses linking directional selection and development stability.

Hypotheses	Definition/processes	Effect of selection in opposite direction	Developmental instability and trait size	Studies supporting hypothesis	Studies contradicting hypothesis
Decanalization	Canalization: organism's or trait's ability to suppress phenotypic variation due to genetic or environmental disturbances* (Waddington, 1957; Scharloo, 1991; Wagner <i>et al.</i> , 1997)	Any selection away from the 'wild type' should decrease canalization and therefore increase phenotypic variation. Developmental stability is expected to decrease in both direction of selection	No particular relationship or U-shaped relationship	Kindred (1967) and Scharloo (1991)	
Genetic-developmental model	Combined effect of selection on trait size and sensitivity to developmental noise (Gavriliets & Hastings, 1994)	Selection for an increasing trait size increases the trait's sensitivity to developmental noise and therefore decreases its developmental stability. Selection for a decreasing trait size provokes the opposite effects	Positive genetic correlation between trait size and developmental instability, but only weak phenotypic correlation†	Bradley (1980)† and Polak <i>et al.</i> (2004)§	Polak & Starnmer (2001)
Developmental homeostasis	The trade-off between growth rate and the ability to correct errors due to developmental noise provokes a decrease in developmental stability for traits under directional selection for increasing trait size (or organism size) (Calow, 1982; Arendt, 1997)	Decrease in developmental stability only for increasing growth rate; opposite response for reduced trait size	Positive relationship: larger traits or fast growing organisms are more unstable	Leamy & Atchley (1985)† and Robinson & Wardrop (2002)**	Leamy (1986) and Shkarad <i>et al.</i> (2001)
5-Homozygosity	Because homozygous individuals are more developmentally instable (Lerner, 1954; Mitton & Grant, 1984; Vøllestad <i>et al.</i> , 1999), directional selection that decreases genetic variation, should indirectly decrease developmental stability	The decrease in heterozygosity does not depend of the direction of selection. Therefore, we expect a decrease in developmental stability regardless of the selection direction	U-shape: both extremes (large and small trait size) should show higher developmental instability	Leamy (1986)	Radwan & DREWNIAK (2001)

Only experimental studies that explicitly support or refute the hypotheses have been reported except when noticed differently.

*The idea of canalization can be expanded to the hypothesis of an optimal growth rate.

†Decrease in FA over generations associated with a decrease in chaete number in the control line but not in the stabilizing selection line.

‡Positive genetic correlation between positional FA and trait size.

§No genetic correlation between trait size and positional-FA in bristle characters.

**Suggest also that developmental instability increases in both up and down lines because the part of the phenotypic variation explain by FA increases in both lines.

**Note that this study does not really test this hypothesis, since it mainly compares FA in fish growing in optimal (high growth), and suboptimal (low growth) conditions. Furthermore the results seem to strongly depend on a few outliers (see Fig. 3). Additionally, there is apparently condition dependent directional asymmetry (see sign of mean asymmetry in Table 2).

are expected to share common genetic and environmental background, and departures from perfect bilateral symmetry are expected to represent the effects of developmental noise and developmental stability (Palmer *et al.*, 1993). On the other hand, directional asymmetry, when one side is always larger than the other (e.g. fallow deer antlers, Pélabon & Joly, 2000) or antisymmetry, when the two sides are always different but without a predictable direction to the differences (Palmer, 2004, 2005), are genetically determined, and are presumably not related to developmental stability (Palmer, 1994). Paradoxically, despite strong evidence that fixed asymmetries are under genetic control, attempts at selecting directional asymmetry have proved unsuccessful in *Drosophila* bristle, ocelli, eye size and chaetae (Smith & Sondhi, 1960; Coyne, 1987; Tuinstra *et al.*, 1990; Monedero *et al.*, 1997).

The genetic control of developmental stability remains poorly understood, and despite empirical evidence suggesting that developmental stability can evolve (Clarke & McKenzie, 1987), attempts to find genetic variance in developmental stability have for the most part proved unsuccessful (Fuller & Houle, 2003; Pélabon *et al.*, 2004 and references therein). Understanding the relationship between directional selection and developmental stability may therefore provide insight into the genetic control of developmental stability, and may help us to better understand the variational properties of organisms.

We conducted artificial selection in opposite directions on a selection index involving the positions of several veins of the *Drosophila melanogaster* wing and tested whether developmental stability, estimated by the level of fluctuating asymmetry, was affected by the selection regime. Because directional asymmetry in wing shape has been observed in *Drosophila* (Klingenberg *et al.*, 1998; Klingenberg & Zaklan, 2000), our selection to change the position of some veins may also affect the directional components of the asymmetry. Therefore we also analysed the effects of selection on directional asymmetry.

Methods

Wing imaging and selection procedure

The *D. melanogaster* used in this selection experiment are descendants of 400 flies collected by L. Harshman in central California in 1991. In 1995, 2000 of these flies were used to found a subpopulation (Lhm) maintained by W.R. Rice until 2004 and D. Houle thereafter. Flies were maintained under a 12 : 12 L : D cycle at 25 °C. Truncated index-selection (combination of two characters of the wing, see below) was performed for both an increase and a decrease in the index value. Wing measurements were obtained on live flies using an automated image-analysis system (WINGMACHINE, Houle *et al.*, 2003), allowing us to measure each fly and carry out the selection procedure. In brief, the wing of a

live fly was immobilized between a slide and a cover slip using a simple suction device, the wing grabber (see Fig. 1 in Houle *et al.*, 2003). The wing was then placed under a microscope equipped with a digital camera and a picture was taken. The positions of several landmarks corresponding to the major intersections of the veins (Fig. 1) were subsequently obtained using a cubic B-spline (Lu & Milios, 1994) that describes all the wing veins distal to a line defined by user-supplied landmarks (dashed line Fig. 1). For each image, an a priori B-spline model is adjusted to the image of the wing using the pixel brightness of the reversed and filtered image (Houle *et al.*, 2003). The same B-spline model was used to adjust images of the right and left wings, images of the right wing being flipped before analysis.

From the landmark position, two traits were defined and combined to calculate the selection index. The first trait, corresponding to the width of the area between the two longest veins (veins 2 and 3), and further referred to as *distance 2–3*, was estimated as follows: 10 evenly spaced points were selected along the fitted spline from landmarks 14 to 3 (landmark 3 included, but not 14). Then the closest point on the opposite vein (landmarks 13–2) to each point was chosen and the distance between the two points was calculated. The average of these 10 distances was then divided by the square root of the area of the wing given by the B-spline function. So the first trait composing the selection index is:

$$I_1 = \frac{\text{average distance between veins 2 and 3}}{\sqrt{\text{total wing area}}}$$

The second trait, corresponding to the relative position of the posterior crossvein, and further referred to as *crossvein position*, was defined as follow:

$$I_2 = (d[9, 12]/d[9, 2] + d[10, 11]/d[1, 10])/2,$$

where $d[a, b]$ is the linear distance between the landmarks a and b . The two traits I_1 and I_2 displayed different phenotypic variances. In order to perform truncated selection of equal strength on the two traits simultaneously, we built a selection index: $I = 2.6 \times I_1 + I_2$ where the coefficient 2.6 was used to account for the difference in phenotypic variance between the two traits.

Starting during early spring 2004, we conducted two selection treatments in opposite directions. The downward selection (referred to as *Down* selection) aimed at decreasing the index value, and resulted in a decrease in the distance between the veins 2 and 3 and a movement of the posterior crossvein towards the proximal end of the wing. In the upward selection (*Up* selection, increasing index value), we selected for an increasing distance between the veins 2 and 3 and a movement of the posterior crossvein towards the distal end of the wing (Fig. 1).

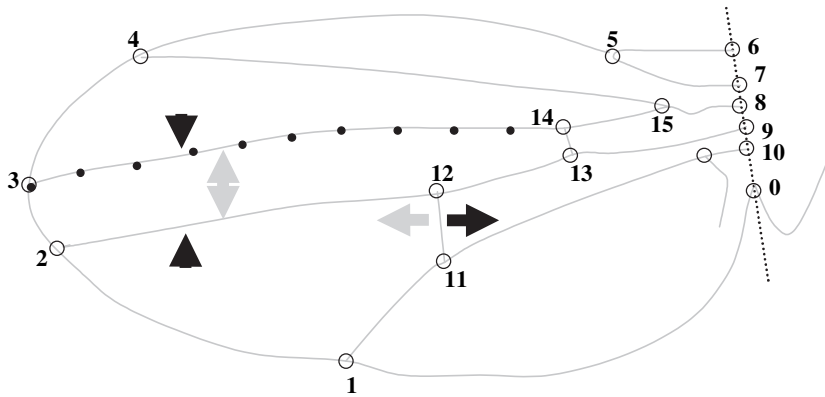


Fig. 1 Representation of a wing of *D. melanogaster* with the reference number of the landmarks used in this study. Effects of selection on the distance between the veins 2 and 3 and on the position of the posterior crossvein are represented (down: black arrows, up: grey arrows). The landmarks 1–5 and 11–14 are used to define the linear distances analysed in this study.

In each generation the left wing of 100 males and 100 females were imaged and their selection-index scores were estimated. For the up (and down) line the 25 males and females with the highest (or lowest) within-gender scores were selected for mating. Five selected individuals from each gender were randomly chosen and combined (in narrow-shell vials: 95 mm height, 25 mm diameter with corn-meal medium) to produce 5 vials with 10 flies per vial. These flies were transferred to new vials after approximately 24 h and the individuals for the next generation were collected as virgins from these vials approximately 8–9 days later. For the control lines, 25 individuals of each gender, haphazardly chosen, were imaged and all were mated (no selection) in the same manner to produce 10 individuals per vial. Each line, up and down selection and control was entirely replicated using individuals from the same initial population (Lhm). In the following, Lhm-1 and Lhm-2 designate the two replicates. In each of these replicates, we defined three selection lines corresponding to the up selection, the down selection, and the control (no selection). For logistic reasons, we estimated FA on males from the generation 9 in Lhm-1 and generation 8 in Lhm-2.

Asymmetry measurements, measurement variance and analysis

We analysed the effect of selection on the patterns of fluctuating and directional asymmetry in the selection index and in several additional traits including two traits representing the size of the wing (length and width) and 36 additional linear distances between different landmarks (see Appendix 1 for traits definition). Unsigned fluctuating asymmetry is the mean of the absolute difference between the left and the right side multiplied by 100 for ease of reading: $FA = 100 \times |L - R|$. Directional asymmetry is estimated as the mean of the signed asymmetry: $DA = 100(L - R)$. We found no correlation between trait size and FA among individuals within traits, or across traits (not shown). Therefore, no transformations of FA measurements were done. For each

trait, including the selection index, we visually inspected the distribution of signed difference ($L - R$) in order to detect outliers. Outliers resulting from the wing being slightly folded or damaged or from a badly fitted spline, were removed. None of the directional asymmetries were correlated with trait size (Lhm-1: $\max r^2 = 0.006$; Lhm-2: $\max r^2 = 0.017$). Estimations of FA were therefore obtained by removing the mean signed difference $L - R$ (directional component of the asymmetry) from each individual asymmetry (Graham *et al.*, 1998). Before correction, directional asymmetry inflated the estimation of FA of 2.2% on average ($\max = 11\%$).

We conducted repeated measurements on *ca.* 30 flies in each line of each replicate. Wings were photographed a first time. Individuals were stored overnight and a new set of picture was taken the next day. Unequal sample size resulted from wings damaged during the storage period. Measurement variances (σ_m^2) estimated from these repeated measurements are presented in Appendix 2. Correction of the FA mean and variance for measurement error were performed as explained in Table 2. Because differences in measurement error appeared across lines and replicates (Appendix 2), corrections were conducted separately for each line within replicate using the measurement variance estimated for the particular line and replicate. Eight traits, however, had large measurement variances that prevented us from estimating the corrected level of FA for some selection lines (see Appendix 3). When these traits were discarded, measurement error inflated FA by 23% on average ($SE = 0.86\%$). These traits were excluded from all analyses.

Analyses of developmental stability in complex organs such as insect wings or mammalian skulls have increasingly made use of procrustes superimposition methods (Klingenberg & McIntyre, 1998; Klingenberg & Zaklan, 2000; Willmore *et al.*, 2005). Although very powerful for estimating variation in the whole structure, this technique presents some difficulties for localizing variation of particular parts of the wing (e.g. 'Pinocchio effect' Chapman, 1990 cited in Klingenberg & McIntyre, 1998;

Table 2 Descriptive statistics for directional and fluctuating asymmetry in the selection index and in the two traits that compose this index.

Trait	Line	Rep.	Trait size		Signed-FA			Unsigned-FA			
			Mean	Mean (95% CI)	Variance	Kurtosis	ME (σ_m^2)	Mean	Variance	CV(FA)	
Selection index	Down	1	0.972	0.119 (-0.034; 0.271)	1.648	0.29	0.566	0.789	0.447	0.848	
		2	0.984	-0.158 (-0.301; -0.109)	1.260	-0.10	0.576	0.643	0.249	0.776	
	Control	1	1.032	0.186 (0.105; 0.333)	1.172	0.21	0.204	0.758	0.390	0.824	
		2	1.047	-0.056 (-0.184; 0.134)	1.120	0.33	0.353	0.692	0.285	0.771	
	Up	1	1.100	0.003 (-0.176; 0.060)	1.343	0.39	0.529	0.710	0.304	0.777	
		2	1.096	-0.000 (-0.067; 0.1187)	0.933	0.13	0.196	0.704	0.237	0.691	
Distance 2-3	Down	1	0.155	0.057 (0.035; 0.091)	0.056	0.29	0.021	0.145	0.016	0.871	
		2	0.156	0.028 (0.017; 0.054)	0.044	0.51	0.025	0.098	0.009	0.945	
	Control	1	0.165	0.052 (0.021; 0.082)	0.039	-0.49	0.016	0.124	0.008	0.704	
		2	0.165	-0.031 (-0.068; 0.009)	0.049	0.13	0.018	0.140	0.012	0.771	
	Up	1	0.174	0.042 (0.001; 0.062)	0.046	0.27	0.029	0.103	0.008	0.851	
		2	0.171	0.031 (0.021; 0.048)	0.041	0.18	0.014	0.134	0.009	0.706	
Crossvein position	Down	1	0.570	-0.031 (-0.180; 0.098)	1.175	0.34	0.225	0.743	0.395	0.845	
		2	0.577	-0.229 (-0.293; -0.227)	0.933	-0.23	0.306	0.650	0.200	0.688	
	Control	1	0.603	0.052 (0.008; 0.246)	0.976	0.49	0.123	0.750	0.280	0.705	
		2	0.617	0.024 (0.024; 0.096)	0.818	0.32	0.203	0.616	0.235	0.787	
	Up	1	0.647	-0.097 (-0.230; -0.041)	0.881	0.28	0.297	0.590	0.238	0.827	
		2	0.651	-0.095 (-0.224; 0.015)	0.784	0.18	0.128	0.644	0.246	0.771	

Fluctuating asymmetry (absolute value of signed-FA) is corrected for directional asymmetry. Mean and variance in FA are corrected for measurement error as follow: $FA_{corr} = \sqrt{FA_{obs}^2 - 2\sigma_m^2/\pi}$ where FA_{obs} is the observed unsigned-FA, and σ_m^2 is the measurement variance calculated as $Var(m_1 - m_2)$, where m_1 and m_2 are the signed-FAs calculated from the first and second measurements. Variance in FA corrected for measurement error is obtained by removing $\sigma_m^2(1 - 2/\pi)$

from the observed variance in FA; see Pélabon *et al.* (2004) for further details. Tests for the difference in FA among treatments on square-root transformed data: selection index, Lhm-1: $F_{2,430} = 1.05$, $P = 0.35$; Lhm-2: $F_{2,398} = 0.77$, $P = 0.46$; distance 2-3, Lhm-1: $F_{2,430} = 0.37$, $P = 0.69$; Lhm-2: $F_{2,398} = 0.22$, $P = 0.80$; crossvein distance, Lhm-1: $F_{2,432} = 1.05$, $P = 0.35$; Lhm-2: $F_{2,398} = 2.17$, $P = 0.12$. Data in bold correspond to traits showing significant directional asymmetry (zero not included in the 95% CI of the mean signed-FA) consistent among replicates.

Walker, 2000; Richtsmeier *et al.*, 2005; Willmore *et al.*, 2005). Indeed, the generalized least-squares algorithm used to adjust the superimposition tends to spread variation from the most variable landmarks to the others (Klingenberg & McIntyre, 1998). Because our selection procedure was expected to affect the position of some landmarks more than others, we did not use procrustes analysis. We first tested for an effect of selection on the overall level of fluctuating asymmetry using a multivariate analysis of variance (MANOVA) on the complete set of inter-landmark distances with selection lines and replicates as factors. Then, we conducted univariate tests (ANOVA) on each distance separately, accounting for multiple testing. Statistical tests were performed on square-root transformed data (see Pélabon *et al.*, 2004 for justification).

Results

Directional selection and fluctuating asymmetry

The selection index responded strongly to both up and down selection in both replicates (Fig. 2 and Table 2). Wing size differed both between selection lines, and between replicates. In Lhm-1, the length of the wing decreased in both up and down selection lines, while in

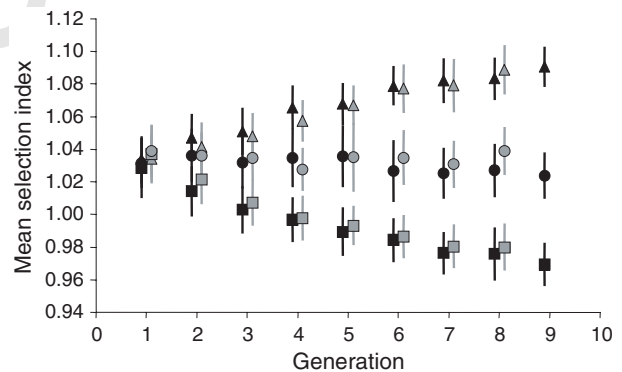


Fig. 2 Values of the mean selection index in the up (triangle), down (square) and control (circle) lines during the first nine (for Lhm-1, black symbols) and eight (for Lhm-2, grey symbols) generations. Bars represent SD.

Lhm-2, wing length was reduced in the down line and increased in the up line (Appendix 1).

The level of FA in the selection index and in the two traits composing this index was extremely low (Table 2), on average 1.06% of the trait size (range: 0.72–1.46%). The corresponding figures for the other wing traits range from 0.18 to 6.52% of the trait size, with an average of

1.37% (average over all traits measured in the different selection lines and replicates). Due to the generally low phenotypic variation in wings, FA still accounted for a range of 0.7–49.0% and an average of 10% of the phenotypic variance in the different wing characters. This is comparable to an average of 14.8% total, and 10.1% for quantitative characters, found in a meta-analysis of fluctuating asymmetry in wild populations (Hansen *et al.*, 2006).

Differences in FA in the selection index between selection lines were small and inconsistent across replicates (Fig. 3a; Table 2) as were the differences in FA in the two traits composing this index (Fig. 3b,c and Table 2). Similarly, differences in the level of FA in other wing traits were of small magnitude and predominantly random (Fig. 4). However, MANOVA revealed differences between the selection lines in the level of FA, similar in both replicates (Fig. 5). These differences are due to a tendency for FA to decrease in both up-selected lines (Fig. 5). This is further revealed by the significantly negative difference in FA between the up-selected lines and the control lines (Fig. 5). In two traits, associated with the position of the posterior crossvein (traits 21 and 26), univariate analysis of variance revealed a statistically significant difference in the level of FA across selection lines, with a lower level of FA in the up lines compared with the down lines, the control lines showing an intermediate level of FA (bold lines in Fig. 4). Other dimensions associated with landmarks 11 and 12 showed similar patterns, though noisier (not shown).

Directional selection and directional asymmetry

Consistent, statistically significant directional asymmetries occur in the two traits composing the selection index across replicates (Table 2) as well as in several other wing traits (Appendix 2). In general these directional asymmetries consisted of traits being larger on the left side than on the right side. Note, however, that the two traits representing the length and the width of the wing did not show any significant pattern of directional asymmetry (Appendix 2). This pattern of directional asymmetry is comparable with the pattern reported by Klingenberg *et al.* (1998), with the left wing slightly wider than the right one (larger distance between landmarks 1 and 5; see Fig. 1a in Klingenberg *et al.*, 1998). The tip of the wing also displays directional asymmetry, although comparison of this pattern is more difficult due to the different methods used in both studies. The two traits composing the selection index showed directional asymmetry in opposite directions, and these effects cancelled each other in the selection index.

Selection affected directional asymmetry independently of the direction of the selection. Indeed, patterns of directional asymmetry were more similar between selected lines within and across replicates, than between control lines across replicates or between selected and

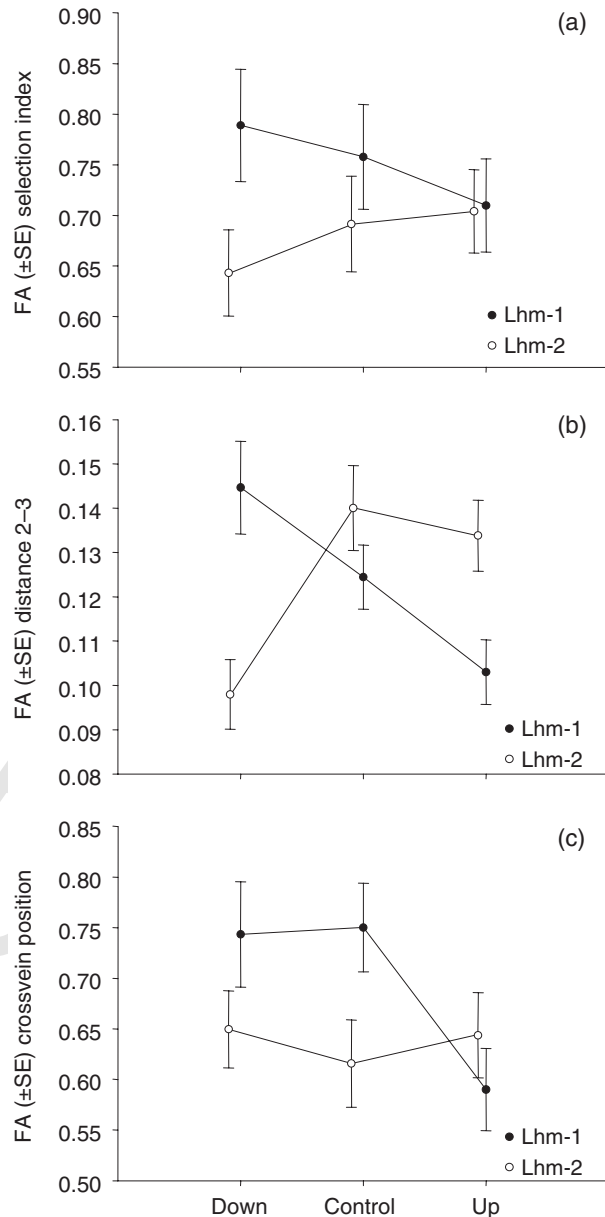


Fig. 3 Mean (\pm SE) FA corrected for measurement error in the different selected lines for (a) the selection index, (b) the distance between veins 2 and 3 and (c) the position of the posterior crossvein (see Table 2 for statistics). Data are in 10^{-2} mm.

control lines within replicates (Table 2 for traits comprising the selection index and Fig. 6 for other wing traits). Furthermore, differences in directional asymmetry in wing traits between control and selected lines were congruent between treatments within replicates (Fig. 7). This indicates that the changes in directional asymmetry provoked by the up selection tended to be similar in direction, and to some extent in magnitude, to the changes provoked by the down selection. We found no

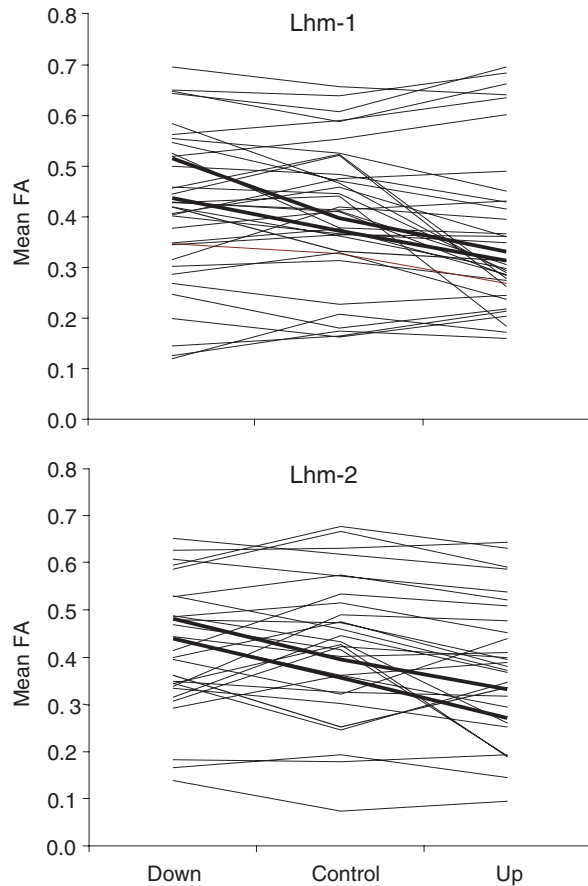


Fig. 4 Mean FA corrected for measurement error for the different wing traits in the different selected lines in the two replicates. Bold lines correspond to traits 21 and 26 that show statistically significant changes in their level of FA in both replicates. Trait 21: line effect: $F_{2,833} = 12.34$, $P < 0.001$, replicate effect: $F_{1,833} = 0.06$, $P = 0.81$, interaction: $F_{2,833} = 0.07$, $P = 0.94$; trait 26: line effect: $F_{2,832} = 9.29$, $P < 0.001$, replicate effect: $F_{1,832} = 0.46$, $P = 0.50$, interaction: $F_{2,832} = 1.38$, $P = 0.25$. Data are in 10^{-2} mm.

correlation among traits between changes in directional asymmetry and changes in FA (average $r = 0.02$).

Discussion

Directional selection and fluctuating asymmetry

Less than 10 generations of index selection on the *D. melanogaster* wing produced pronounced changes in the positions of the two longest veins and the posterior crossvein. The mean of the selection index was changed more than 3 SD away from the original mean in both up and down lines. Despite this marked response to selection, changes in the level of FA in the selection index or the two traits composing this index were small and inconsistent across replicates. The effects of selection on the level of FA in other wing characters were generally

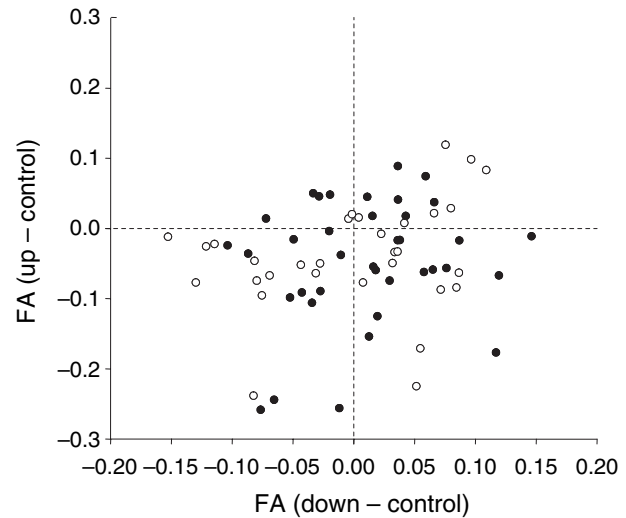


Fig. 5 Differences in FA (corrected for measurement error) in wing traits between selected and control lines (black dots: Lhm-1; open dots: Lhm-2). Data are in 10^{-2} mm. Statistically significant differences among selection lines in the overall level of FA are revealed by the multivariate analysis of variance (MANOVA: replicates \times selection lines: Wilk's $\lambda = 0.9575$, $F_{30,787} = 1.16$, $P = 0.25$; replicates: Wilk's $\lambda = 0.9583$, $F_{30,787} = 1.14$, $P = 0.27$; selection lines: Wilk's $\lambda = 0.9055$, $F_{30,787} = 2.74$, $P < 0.001$; note that there was not evidence of heterogeneity in the covariance structure among groups). Mean difference in FA across traits between selected lines (95% CI obtained by bootstrap analysis): Lhm-1, down - control = 0.87 (-1.03; 2.79); up - control = -4.60 (-7.96; -2.44); Lhm-2, down - control = -0.20 (-2.80; 2.16); up - control = -4.01 (-6.98; -1.46).

small, but we found a tendency for a decrease in FA in the up-selected lines. This tendency was more marked for a few traits associated with the position of the posterior crossvein. Although one can suggest that eight or nine generations of selection were insufficient to significantly affect developmental stability, the large changes in the wing traits themselves suggest strong effects on the genetics of the traits. Therefore, our results are evidence against a strong effect of directional selection on developmental stability. If anything, we see an increase in developmental stability in the two up lines, indicating that directional selection may also act as a canalizing force. Indeed, varied effects of directional selection on canalization are not unexpected. As directional selection moves the population in genotype space, the local curvature of the genotype-phenotype map will determine whether phenotypic changes becomes canalized or decanalized (Hansen & Wagner, 2001; Hermisson & Wagner, 2004; Carter *et al.*, 2005).

Very few studies have experimentally tested the hypothesis that directional selection reduces developmental stability. A review of these studies shows, however, that our results are not atypical, and there is only weak, if any, support that directional selection

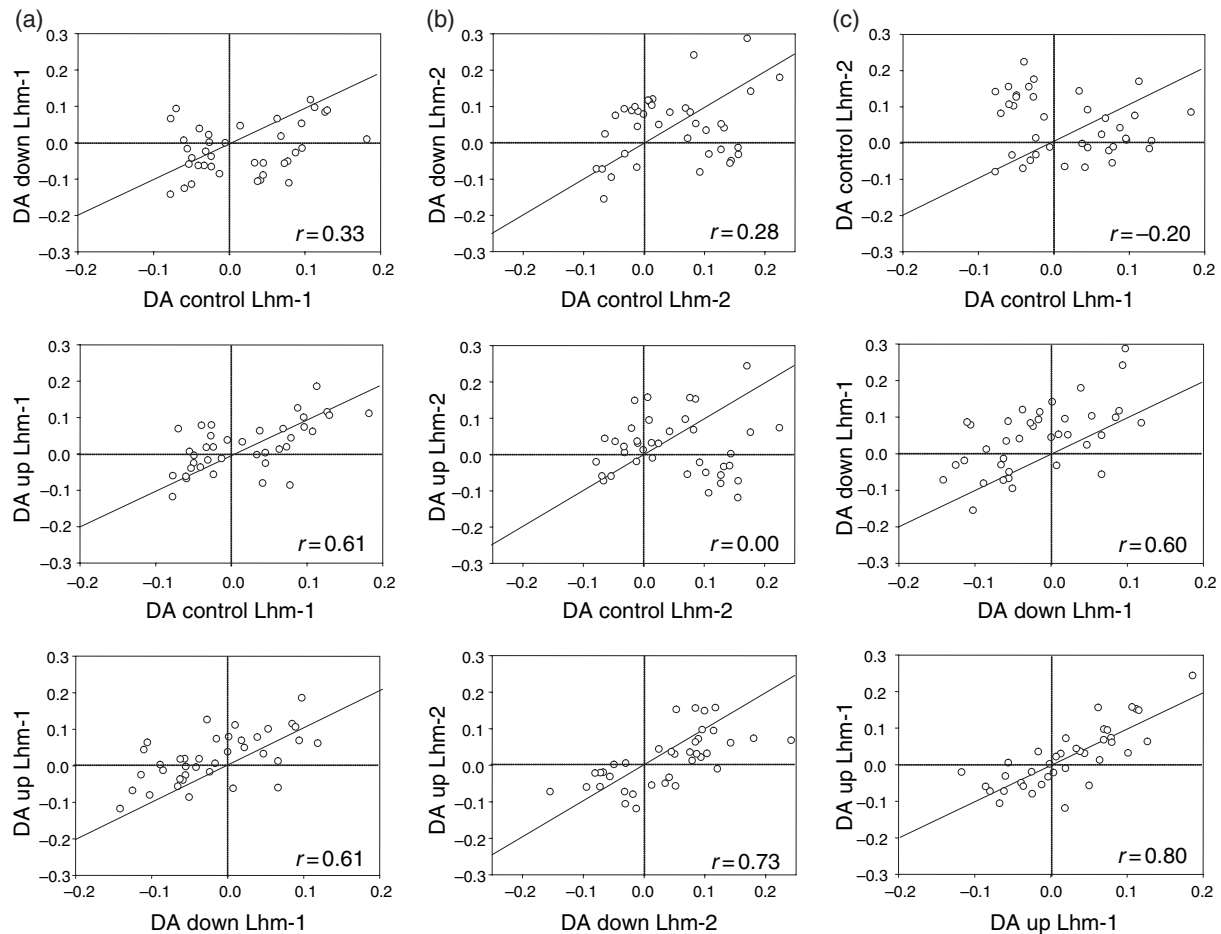


Fig. 6 Relationship in the pattern of directional asymmetry in wing traits between selected and control lines within and among replicates. Coefficients of correlation are given as indicator of the level of congruence between the different treatments. Data are in 10^{-2} mm.

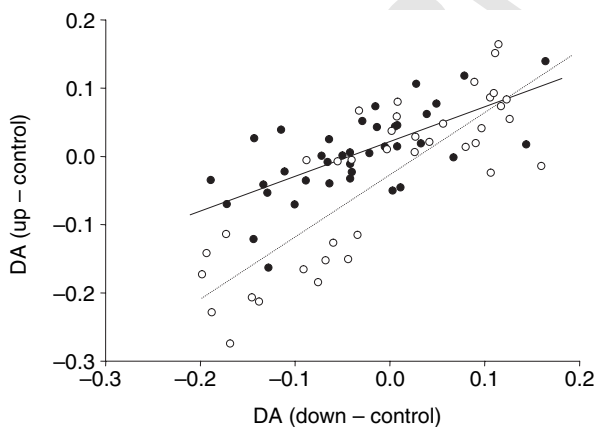


Fig. 7 Differences in directional asymmetry in wing traits between selected and control lines (black dots: Lhm-1; open dots: Lhm-2). Data are in 10^{-2} mm.

decreases developmental stability (Table 1). Furthermore, the only other study, to our knowledge, that measured developmental stability on the trait directly under selection (Leamy, 1986), showed inconsistent results, and the main changes in FA may have resulted from a decrease in heterozygosity.

In the up-selected lines, where the posterior crossvein was moved towards the distal end of the wing, FA tended to decrease, and significantly so for a few traits associated with the position of this crossvein, while FA remained unchanged or slightly increased in the down-selected lines. This asymmetry in the effect of the up and down selection on developmental stability is inconsistent with the different hypotheses listed in Table 1. First, these results do not support the homozygosity model that predicts a decrease in developmental stability regardless of the direction of the selection, since it is the loss of heterozygosity in selected lines that is assumed to affect the level of developmental stability (Table 1).

Furthermore, our design should not have led to a very big difference in homozygosity between selected lines and controls. Second, our results refute the classical canalization model in which a decrease in developmental stability is expected with directional selection, whatever the direction of selection because both up and down selection should represent a departure from the wild (most canalized) type.

In principle, an asymmetrical response of fluctuating asymmetry to selection in opposite direction can be explained by any model that links developmental stability to trait size such as the developmental-homeostasis model (Table 1). This model is, however, unlikely to apply in our study, as we selected on shape characters and did not observe changes in wing size congruent with the changes in developmental stability. Conversely, the weak relationship between directional selection and developmental stability observed here may not apply to selection on trait size.

One can speculate that selection on the position of the posterior crossvein acted to move landmark 11, and to some extends landmark 12, towards areas of the wing possessing a different degree of developmental stability. If true, this hypothesis suggests that changes in fluctuating asymmetry associated with the changes in trait position will not reflect changes in developmental stability, but may result from different levels of developmental stability in different regions of the body. The movement of the posterior crossvein within these regions, as a response to selection, could therefore produce changes in the observed level of fluctuating asymmetry without affecting the developmental stability of the wing. Further studies are clearly needed to confirm or refute this hypothesis.

Directional selection and directional asymmetry

Selection in opposite directions caused systematic changes in directional asymmetry, resulting in congruent patterns in directional asymmetry in all selected lines. Despite strong evidence for genetic control of directional asymmetry (Palmer, 2004), experiments that selected for directional asymmetry in populations displaying fluctuating asymmetry have been unsuccessful (Smith & Sondhi, 1969; Coyne, 1987; Tuinstra *et al.*, 1990). These results therefore suggest that traits displaying fluctuating asymmetry are generally void of additive genetic variation for directional asymmetry, as suggested by Monedero *et al.* (1997). However, remarkably few if any studies have selected for an increase or decrease in existing directional asymmetry. The question therefore remains whether selection on traits displaying directional asymmetry can increase or decrease the level of directional asymmetry.

In the presence of additive genetic variation for directional asymmetry, one might expect that the response to selection in the position of the veins would utilize this genetic variance. In this case, we would

predict that selection in opposite directions would have opposite effects on directional asymmetry. For example, if selection on the left wing for a more distal position of the posterior crossvein would provoke an increase in the directional asymmetry ($L > R$) in the distance between landmarks 5 and 11, or 5 and 12, moving the posterior crossvein in the other direction should decrease this directional asymmetry. Surprisingly, in our experiment the changes in directional asymmetry from the control to the selected lines were similar whatever the direction of the selection (up or down, Fig. 7). This created a general pattern of directional asymmetry congruent across selected lines in both replicates. Therefore we can exclude the hypothesis that changes in directional asymmetry correspond to a response to selection on genetic variance in directional asymmetry.

Given that the pattern of directional asymmetry observed in selected lines is weakly expressed in the control lines, one can suggest that the changes are due to the expression of partly hidden genetic variation in directional asymmetry following genetic decanalization of traits under directional selection. Although speculative, this hypothesis could explain why patterns of directional asymmetry do not depend on the direction of selection.

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Appendix 1 Definition and descriptive statistics (mean and variance) for the different traits (inter-landmark distances) of the *D. melanogaster* wing used in the analysis of FA and DA.

Trait	n	Down				Control				Up			
		Lhm-1 147		Lhm-2 140		Lhm-1 147		Lhm-2 130		Lhm-1 147		Lhm-2 144	
		Mean	Var	Mean	Var	Mean	Var	Mean	Var	Mean	Var	Mean	Var
1	Width*	0.395	1.08E-04	0.390	4.51E-04	0.408	1.63E-04	0.399	1.58E-04	0.400	3.52E-04	0.408	1.34E-04
2	Length†	0.854	4.87E-04	0.839	2.08E-03	0.872	7.15E-04	0.863	6.35E-04	0.867	1.45E-03	0.882	6.01E-04
4	1–2	0.367	1.52E-04	0.364	3.87E-04	0.379	1.75E-04	0.376	1.27E-04	0.381	2.93E-04	0.380	1.35E-04
5	1–3	0.439	1.79E-04	0.438	5.24E-04	0.461	2.54E-04	0.458	1.59E-04	0.465	4.05E-04	0.465	1.74E-04
6	1–4	0.432	1.69E-04	0.431	5.74E-04	0.456	2.41E-04	0.447	1.74E-04	0.452	4.76E-04	0.459	1.94E-04
7	1–5	0.480	1.69E-04	0.469	6.74E-04	0.487	2.38E-04	0.473	3.02E-04	0.471	4.87E-04	0.485	1.96E-04
8	1–11	0.178	5.03E-05	0.170	9.83E-05	0.157	1.35E-04	0.147	6.23E-05	0.120	4.50E-05	0.121	3.65E-05
9	1–12	0.224	5.06E-05	0.217	1.48E-04	0.219	8.51E-05	0.205	8.35E-05	0.197	1.08E-04	0.204	5.25E-05
10	1–13	0.369	1.19E-04	0.357	4.07E-04	0.364	1.46E-04	0.348	2.47E-04	0.344	2.84E-04	0.361	1.25E-04
11	1–14	0.386	1.17E-04	0.374	4.49E-04	0.382	1.57E-04	0.366	2.40E-04	0.363	3.09E-04	0.380	1.29E-04
12	2–3	0.094	1.50E-05	0.096	2.53E-05	0.109	3.55E-05	0.105	1.50E-05	0.113	2.71E-05	0.113	2.37E-05
13	2–4	0.251	5.04E-05	0.255	1.44E-04	0.273	1.14E-04	0.263	5.77E-05	0.270	1.20E-04	0.273	8.67E-05
14	2–5	0.696	3.10E-04	0.683	1.30E-03	0.711	4.69E-04	0.701	4.28E-04	0.705	9.59E-04	0.716	3.85E-04
15	2–11	0.472	1.66E-04	0.459	6.20E-04	0.458	2.40E-04	0.449	1.83E-04	0.433	3.77E-04	0.433	1.69E-04
16	2–12	0.452	1.53E-04	0.439	5.44E-04	0.445	2.57E-04	0.428	2.29E-04	0.419	4.40E-04	0.426	1.90E-04
17	2–13	0.628	2.79E-04	0.612	1.08E-03	0.628	3.66E-04	0.612	4.07E-04	0.615	7.67E-04	0.631	2.88E-04
18	2–14	0.629	2.62E-04	0.613	1.07E-03	0.630	3.61E-04	0.614	3.93E-04	0.617	7.59E-04	0.633	2.90E-04
19	3–4	0.202	3.98E-05	0.206	1.03E-04	0.214	7.02E-05	0.209	5.59E-05	0.210	7.88E-05	0.211	6.10E-05
20	3–5	0.710	3.20E-04	0.698	1.39E-03	0.727	4.79E-04	0.720	4.28E-04	0.723	9.97E-04	0.734	3.89E-04
21	3–11	0.519	1.78E-04	0.508	7.62E-04	0.515	2.74E-04	0.506	2.06E-04	0.497	4.69E-04	0.496	1.96E-04
22	3–12	0.486	1.61E-04	0.475	6.45E-04	0.485	2.80E-04	0.469	2.36E-04	0.462	4.97E-04	0.468	2.04E-04
23	3–13	0.657	2.92E-04	0.642	1.21E-03	0.661	3.99E-04	0.647	4.12E-04	0.650	8.31E-04	0.666	2.96E-04
24	3–14	0.653	2.75E-04	0.639	1.18E-03	0.657	3.85E-04	0.644	3.97E-04	0.647	8.10E-04	0.662	2.95E-04
25	4–5	0.552	2.69E-04	0.537	1.10E-03	0.562	3.87E-04	0.559	4.22E-04	0.560	8.44E-04	0.573	3.31E-04
26	4–11	0.437	1.64E-04	0.426	7.21E-04	0.439	2.28E-04	0.431	2.20E-04	0.430	4.89E-04	0.435	1.90E-04
27	4–12	0.379	1.36E-04	0.367	5.40E-04	0.378	2.04E-04	0.365	2.10E-04	0.360	4.38E-04	0.370	1.71E-04
28	4–13	0.526	2.45E-04	0.508	1.01E-03	0.525	3.31E-04	0.514	4.02E-04	0.516	7.41E-04	0.534	2.68E-04
29	4–14	0.515	2.26E-04	0.498	9.46E-04	0.514	3.16E-04	0.503	3.86E-04	0.504	7.05E-04	0.522	2.61E-04
30	5–11	0.304	9.62E-05	0.300	3.35E-04	0.330	1.98E-04	0.326	1.67E-04	0.352	2.94E-04	0.364	1.32E-04
31	5–12	0.267	8.67E-05	0.264	2.46E-04	0.285	1.16E-04	0.291	1.10E-04	0.303	1.82E-04	0.308	9.81E-05
32	5–13	0.124	1.78E-05	0.122	5.50E-05	0.130	2.80E-05	0.131	2.52E-05	0.132	4.85E-05	0.130	3.15E-05
33	5–14	0.098	1.56E-05	0.098	3.34E-05	0.105	2.53E-05	0.108	1.97E-05	0.108	3.26E-05	0.106	2.45E-05
34	11–12	0.079	9.55E-06	0.079	3.59E-05	0.086	3.13E-05	0.085	1.86E-05	0.096	2.86E-05	0.098	1.72E-05
35	11–13	0.191	6.99E-05	0.188	1.60E-04	0.208	1.25E-04	0.202	1.30E-04	0.225	1.45E-04	0.243	9.38E-05
36	11–14	0.208	6.55E-05	0.204	1.83E-04	0.226	1.29E-04	0.219	1.26E-04	0.244	1.61E-04	0.259	9.15E-05
37	12–13	0.177	7.89E-05	0.174	1.40E-04	0.184	7.64E-05	0.186	8.95E-05	0.197	8.99E-05	0.206	8.55E-05
38	12–14	0.183	6.96E-05	0.179	1.42E-04	0.190	7.01E-05	0.192	8.17E-05	0.203	8.93E-05	0.212	8.17E-05
39	13–14	0.031	3.00E-06	0.028	7.69E-06	0.031	5.05E-06	0.030	4.93E-06	0.032	6.23E-06	0.031	5.08E-06

Means and variances are given for each selection line in each replicate (Lhm-1 and Lhm-2), *n* corresponds to the number of fly measured in each line. Except noted otherwise, traits are defined by the linear distance between the two given landmarks. All distances are in mm. Trait 3 corresponds to the wing area and is not reported here.

*Wing width is defined by the distance of the line-connecting landmark 1 to the midpoint of the line connecting landmarks 4 and 5.

†The length of the wing is defined by the distance between landmarks 3 and 9. Test for the difference in wing length across treatment, Lhm-1: $F_{2,432} = 15.96$, $P < 0.001$; Lhm-2: $F_{2,400} = 59.18$, $P < 0.001$.

Appendix 2 Summary statistics (mean, SE and measurement variance) for the signed-FA (100[L - R]) in the different traits of the *D. melanogaster* wing (L and R in mm). Measurement variances were estimated as follow: σ_m^2 (FA) = $\text{Var}(m_1 - m_2)$, where m_1 and m_2 are the signed-FAs calculated from the first and second measurements on n repeated measures.

Trait	Down						Control						Up					
	Lhm-1		Lhm-2		Lhm-1		Lhm-2		Lhm-1		Lhm-2		Lhm-1		Lhm-2			
	Mean	SE	σ_m^2 (n = 28)	Mean	SE	σ_m^2 (n = 26)	Mean	SE	σ_m^2 (n = 32)	Mean	SE	σ_m^2 (n = 30)	Mean	SE	σ_m^2 (n = 31)	Mean	SE	σ_m^2 (n = 30)
1	0.018	0.027	0.081	0.095	0.027	0.041	0.068	0.024	0.034	0.069	0.026	0.089	0.070	0.024	0.042	0.097	0.027	0.026
2	-0.085	0.050	0.092	0.012	0.055	0.098	-0.013	0.048	0.076	0.072	0.055	0.099	-0.012	0.043	0.108	-0.054	0.051	0.092
4	-0.103	0.061	0.184	-0.155	0.065	0.280	0.041	0.062	0.290	-0.067	0.067	0.653	-0.080	0.066	0.434	-0.072	0.072	0.366
5	-0.051	0.057	0.170	-0.095	0.057	0.240	0.077	0.058	0.221	-0.054	0.065	0.349	-0.086	0.061	0.204	-0.060	0.062	0.222
6	-0.056	0.052	0.216	-0.067	0.048	0.140	0.045	0.047	0.163	-0.012	0.054	0.266	-0.025	0.049	0.218	-0.019	0.049	0.155
7	0.097	0.049	0.136	0.288	0.049	0.137	0.113	0.047	0.148	0.171	0.048	0.427	0.186	0.046	0.171	0.244	0.051	0.104
8	0.094	0.067	0.189	0.242	0.067	0.350	-0.070	0.062	0.150	0.082	0.059	0.457	0.069	0.058	0.178	0.068	0.056	0.170
9	-0.038	0.055	0.156	0.120	0.050	0.131	-0.024	0.043	0.055	0.014	0.042	0.118	0.019	0.041	0.087	-0.010	0.040	0.049
10	0.039	0.058	0.120	0.180	0.058	0.364	-0.039	0.062	0.149	0.224	0.063	0.498	0.079	0.055	0.301	0.074	0.057	0.115
11	0.001	0.055	0.141	0.142	0.054	0.268	-0.027	0.054	0.165	0.176	0.054	0.371	0.080	0.046	0.162	0.061	0.053	0.166
12	0.066	0.034	0.061	0.050	0.033	0.079	0.063	0.026	0.049	0.024	0.030	0.047	0.013	0.031	0.065	0.031	0.027	0.317
13	-0.066	0.049	0.198	-0.030	0.042	0.098	-0.024	0.043	0.168	-0.032	0.047	0.079	-0.056	0.042	0.148	0.006	0.047	0.177
14	-0.015	0.042	0.111	0.114	0.043	0.080	0.096	0.041	0.065	0.009	0.039	0.047	0.074	0.036	0.080	0.095	0.037	0.083
15	-0.017	0.063	0.114	0.093	0.056	0.115	-0.055	0.050	0.145	-0.033	0.052	0.087	0.007	0.050	0.168	0.022	0.043	0.191
16	-0.106	0.078	0.205	0.079	0.074	0.074	0.037	0.070	0.086	-0.001	0.074	0.057	0.064	0.078	0.262	0.013	0.070	0.169
17	-0.042	0.053	0.130	0.041	0.055	0.216	-0.049	0.054	0.234	0.132	0.061	0.111	-0.004	0.049	0.217	-0.033	0.054	0.216
18	-0.114	0.050	0.159	-0.018	0.055	0.207	-0.050	0.049	0.137	0.127	0.056	0.114	-0.025	0.048	0.133	-0.079	0.055	0.372
19	-0.142	0.055	0.156	-0.072	0.046	0.116	-0.078	0.051	0.171	-0.079	0.053	0.089	-0.117	0.036	0.134	-0.020	0.050	0.133
20	-0.057	0.046	0.076	0.089	0.045	0.086	0.073	0.043	0.043	-0.020	0.046	0.055	0.020	0.036	0.047	0.072	0.038	0.033
21	-0.024	0.059	0.075	0.075	0.056	0.100	-0.031	0.048	0.064	-0.048	0.052	0.091	-0.017	0.044	0.079	0.036	0.039	0.057
22	-0.110	0.076	0.187	0.086	0.072	0.064	0.079	0.069	0.047	-0.010	0.071	0.044	0.044	0.077	0.152	0.031	0.071	0.067
23	-0.059	0.053	0.110	0.035	0.054	0.178	-0.053	0.056	0.165	0.103	0.063	0.121	-0.039	0.048	0.260	-0.050	0.054	0.070
24	-0.125	0.048	0.167	-0.031	0.055	0.168	-0.059	0.051	0.104	0.107	0.060	0.105	-0.068	0.049	0.080	-0.106	0.057	0.234
25	0.053	0.064	0.283	0.103	0.061	0.135	0.095	0.056	0.146	0.013	0.057	0.076	0.101	0.051	0.147	0.032	0.054	0.165
26	0.000	0.052	0.124	0.045	0.049	0.055	-0.005	0.042	0.079	-0.011	0.046	0.084	0.038	0.043	0.098	0.037	0.037	0.071
27	-0.027	0.070	0.202	0.084	0.066	0.054	0.088	0.068	0.086	0.043	0.069	0.093	0.127	0.072	0.149	0.064	0.065	0.114
28	0.022	0.061	0.205	0.052	0.063	0.227	-0.027	0.061	0.139	0.128	0.066	0.120	0.050	0.058	0.377	-0.057	0.059	0.097
29	-0.062	0.061	0.249	-0.013	0.065	0.207	-0.033	0.059	0.158	0.156	0.067	0.162	0.018	0.061	0.157	-0.119	0.061	0.252
30	0.010	0.063	0.095	0.053	0.055	0.129	0.182	0.056	0.086	0.086	0.050	0.104	0.112	0.053	0.127	0.153	0.046	0.061
31	0.118	0.065	0.212	0.084	0.061	0.074	0.107	0.065	0.073	0.076	0.068	0.056	0.062	0.070	0.126	0.156	0.060	0.074
32	0.085	0.028	0.086	0.099	0.030	0.055	0.127	0.031	0.070	-0.015	0.035	0.104	0.115	0.033	0.113	0.149	0.032	0.085
33	0.089	0.028	0.082	0.117	0.030	0.071	0.129	0.028	0.066	0.006	0.032	0.075	0.106	0.028	0.047	0.158	0.029	0.084
34	-0.063	0.026	0.044	-0.073	0.022	0.042	-0.041	0.026	0.057	-0.069	0.028	0.076	-0.036	0.029	0.053	-0.059	0.029	0.077
35	-0.055	0.068	0.100	-0.050	0.065	0.153	0.033	0.059	0.133	0.144	0.061	0.172	-0.002	0.057	0.355	0.002	0.054	0.166
36	-0.089	0.066	0.193	-0.081	0.060	0.126	0.045	0.055	0.087	0.092	0.056	0.138	0.003	0.055	0.116	-0.022	0.050	0.253
37	0.066	0.084	0.236	-0.056	0.074	0.107	-0.077	0.078	0.133	0.142	0.080	0.129	-0.060	0.080	0.326	-0.031	0.074	0.155
38	0.007	0.082	0.304	-0.032	0.069	0.111	-0.060	0.074	0.113	0.156	0.080	0.110	-0.062	0.079	0.188	-0.072	0.072	0.188
39	0.047	0.021	0.062	0.024	0.019	0.016	0.014	0.019	0.046	-0.065	0.021	0.046	0.033	0.021	0.088	0.044	0.022	0.057

Appendix 3 Descriptive statistics (mean, variance and CV) for FA in the wing traits.

Trait	Down						Control						Up					
	Lhm-1			Lhm-2			Lhm-1			Lhm-2			Lhm-1			Lhm-2		
	Mean	Var	CV(FA)	Mean	Var	CV(FA)	Mean	Var	CV(FA)	Mean	Var	CV(FA)	Mean	Var	CV(FA)	Mean	Var	CV(FA)
1	0.125	0.011	0.84	0.197	0.023	0.77	0.175	0.016	0.72	-	-	-	0.159	0.016	0.79	0.208	0.031	0.85
2	0.377	0.122	0.93	0.443	0.128	0.81	0.412	0.093	0.74	0.402	0.117	0.85	0.306	0.066	0.84	0.409	0.105	0.79
4	0.459	0.139	0.81	0.421	0.116	0.81	0.446	0.073	0.60	-	-	-	0.292	0.108	1.12	0.449	0.172	0.92
5	0.428	0.111	0.78	0.396	0.049	0.56	0.413	0.104	0.78	0.320	0.078	0.87	0.431	0.135	0.85	0.440	0.129	0.82
6	0.302	0.076	0.91	0.343	0.063	0.73	0.313	0.058	0.77	0.247	0.033	0.74	0.275	0.045	0.77	0.345	0.073	0.78
7	0.346	0.098	0.91	0.342	0.074	0.79	0.328	0.071	0.81	-	-	-	0.268	0.060	0.92	0.376	0.121	0.93
8	0.554	0.151	0.70	0.401	0.111	0.83	0.524	0.137	0.71	-	-	-	0.450	0.105	0.72	0.383	0.137	0.97
9	0.436	0.093	0.70	0.361	0.078	0.77	0.371	0.081	0.77	0.253	0.044	0.83	0.312	0.059	0.78	0.336	0.068	0.78
10	0.456	0.156	0.87	0.271	0.018	0.50	0.521	0.139	0.72	-	-	-	0.277	0.055	0.84	0.454	0.137	0.82
11	0.427	0.111	0.78	0.290	0.042	0.71	0.408	0.101	0.78	-	-	-	0.283	0.058	0.85	0.378	0.098	0.83
12	0.246	0.042	0.83	0.184	0.036	1.03	0.180	0.019	0.75	0.199	0.024	0.77	0.218	0.022	0.68	-	-	-
13	0.268	0.070	0.99	0.292	0.056	0.81	0.225	0.050	1.00	0.361	0.069	0.73	0.243	0.039	0.82	0.294	0.045	0.72
14	0.286	0.066	0.89	0.334	0.067	0.78	0.329	0.076	0.84	0.302	0.050	0.74	0.238	0.046	0.90	0.253	0.047	0.86
15	0.523	0.194	0.84	0.468	0.101	0.68	0.377	0.084	0.77	0.417	0.080	0.68	0.366	0.055	0.64	0.192	0.041	1.05
16	0.647	0.254	0.78	0.652	0.246	0.76	0.588	0.281	0.90	0.618	0.256	0.82	0.662	0.156	0.60	0.585	0.194	0.75
17	0.418	0.095	0.74	0.315	0.104	1.02	0.360	0.068	0.72	0.445	0.158	0.89	0.298	0.037	0.65	0.368	0.063	0.68
18	0.344	0.090	0.87	0.345	0.084	0.84	0.364	0.079	0.77	0.427	0.098	0.73	0.360	0.072	0.74	0.189	0.021	0.77
19	0.401	0.113	0.84	0.307	0.086	0.95	0.365	0.069	0.72	0.422	0.082	0.68	0.349	0.070	0.76	0.400	0.062	0.62
20	0.348	0.110	0.96	0.347	0.070	0.76	0.375	0.087	0.79	0.325	0.102	0.98	0.286	0.055	0.82	0.317	0.073	0.85
21	0.516	0.169	0.80	0.481	0.101	0.66	0.397	0.110	0.84	0.395	0.096	0.78	0.330	0.084	0.88	0.332	0.054	0.70
22	0.642	0.230	0.75	0.625	0.255	0.81	0.606	0.292	0.89	0.629	0.200	0.71	0.695	0.213	0.66	0.643	0.237	0.76
23	0.428	0.110	0.78	0.336	0.116	1.01	0.440	0.090	0.68	0.489	0.138	0.76	0.184	0.028	0.91	0.477	0.124	0.74
24	0.315	0.061	0.78	0.399	0.093	0.77	0.419	0.096	0.74	0.475	0.128	0.75	0.395	0.101	0.80	0.379	0.078	0.73
25	0.405	0.147	0.95	0.480	0.139	0.78	0.457	0.098	0.69	0.473	0.110	0.70	0.359	0.089	0.83	0.396	0.093	0.77
26	0.418	0.088	0.71	0.440	0.087	0.67	0.331	0.069	0.79	0.356	0.057	0.67	0.314	0.061	0.79	0.272	0.055	0.86
27	0.561	0.200	0.80	0.607	0.176	0.69	0.589	0.237	0.83	0.571	0.181	0.75	0.635	0.178	0.66	0.538	0.192	0.82
28	0.443	0.143	0.85	0.413	0.156	0.96	0.520	0.137	0.71	0.535	0.148	0.72	0.261	0.040	0.76	0.509	0.135	0.72
29	0.403	0.132	0.90	0.485	0.132	0.75	0.475	0.128	0.75	0.517	0.137	0.72	0.489	0.132	0.74	0.453	0.075	0.61
30	0.547	0.171	0.76	0.440	0.092	0.69	0.471	0.152	0.83	0.360	0.079	0.78	0.414	0.102	0.77	0.389	0.081	0.73
31	0.519	0.132	0.70	0.529	0.164	0.77	0.552	0.236	0.88	0.573	0.194	0.77	0.602	0.211	0.76	0.521	0.170	0.79
32	0.119	0.016	1.06	0.181	0.034	1.01	0.206	0.027	0.80	0.177	0.020	0.80	0.170	0.017	0.76	0.193	0.024	0.81
33	0.145	0.014	0.83	0.166	0.026	0.97	0.165	0.022	0.90	0.194	0.020	0.72	0.213	0.023	0.71	0.144	0.011	0.73
34	0.199	0.017	0.65	0.114	0.010	0.88	0.163	0.015	0.75	0.116	0.011	0.92	0.204	0.028	0.83	0.136	0.021	1.06
35	0.583	0.222	0.81	0.529	0.154	0.74	0.466	0.153	0.84	0.457	0.086	0.64	0.289	0.028	0.58	0.371	0.114	0.91
36	0.499	0.187	0.87	0.487	0.123	0.72	0.483	0.120	0.72	0.432	0.074	0.63	0.429	0.124	0.82	0.262	0.035	0.72
37	0.695	0.290	0.77	0.595	0.283	0.89	0.657	0.328	0.87	0.677	0.329	0.85	0.641	0.176	0.66	0.631	0.232	0.76
38	0.649	0.242	0.76	0.586	0.207	0.78	0.638	0.266	0.81	0.665	0.251	0.75	0.683	0.230	0.70	0.591	0.199	0.75
39	0.052	0.000	0.24	0.138	0.013	0.81	0.085	0.001	0.31	0.072	0.003	0.70	-	-	-	0.094	0.004	0.67

Fluctuating asymmetry is calculated as $100 \times |L - R|$ where L and R are in mm. Data are corrected for directional asymmetry and measurement error as described in Table 2 [see Pélabon *et al.* (2004) for further details]. Missing values correspond to traits where the ME was too large to properly estimate the corrected FA.

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