EVOLUTION OF VARIATION AND VARIABILITY UNDER FLUCTUATING, STABILIZING, AND DISRUPTIVE SELECTION

Christophe Pélabon,[1,2] Thomas F. Hansen,[3,4] Ashley J. R. Carter,[3,5] and David Houle[3]

[1]Department of Biology, Center for Conservation Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

  [2]E-mail: christophe.pelabon@bio.ntnu.no

[3]Department of Biological Science, Florida State University, Tallahassee, Florida 32306

[4]CEES, Department of Biology, University of Oslo, PO Box 1066, 0316 Oslo, Norway

[5]Department of Biological Sciences, California State University, Long Beach, California 90840

How variation and variability (the capacity to vary) may respond to selection remain open questions. Indeed, effects of different selection regimes on variational properties, such as canalization and developmental stability are under debate. We analyzed the patterns of among- and within-individual variation in two wing-shape characters in populations of *Drosophila melanogaster* maintained under fluctuating, disruptive, and stabilizing selection for more than 20 generations. Patterns of variation in wing size, which was not a direct target of selection, were also analyzed. Disruptive selection dramatically increased phenotypic variation in the two shape characters, but left phenotypic variation in wing size unaltered. Fluctuating and stabilizing selection consistently decreased phenotypic variation in all traits. In contrast, within-individual variation, measured by the level of fluctuating asymmetry, increased for all traits under all selection regimes. These results suggest that canalization and developmental stability are evolvable and presumably controlled by different underlying genetic mechanisms, but the evolutionary responses are not consistent with an adaptive response to selection on variation. Selection also affected patterns of directional asymmetry, although inconsistently across traits and treatments.

KEY WORDS: Canalization, directional asymmetry, disruptive selection, *Drosophila melanogaster*, fluctuating asymmetry, fluctuating selection, stabilizing selection, variational properties.

Phenotypic variation and the relative contribution of its different components vary enormously across populations and characters (Houle 1998). Traditionally, attempts at explaining this diversity have focused on patterns of selection and levels of mutation. More recent work has investigated the possibility that variational potential itself may be an evolvable character. This work stemmed from Wagner and Altenberg's (1996) conceptual distinction between variation and variability, where the latter refers to variational potential, that is, the ability to vary. Population variation has both genetic and environmental components. These are affected by the organism's capacity to respond to or regulate the effects of various internal (genetic) or external (environmental) differences. Genetic variation is caused by genotypic differences among individuals. Environmental variation is caused by exposure to different macro- or microenvironments, as well as stochastic perturbations during the development that results in developmental noise (Nijhout and Davidowitz 2003). Organisms may differ in their response to genetic or environmental differences, some being relatively robust and able to produce similar phenotypes in the face of genetic (mutational) or environmental changes, and others being more

sensitive, or perhaps plastic, in their responses. The variability of an organism or genotype is thus determined by its sensitivity or capability to respond to genetic and environmental differences. Traditionally, the buffering of genetic and environmental differences between individuals have been referred to as genetic and environmental canalization, respectively (Wagner et al. 1997; Flatt 2005), whereas mechanisms buffering against stochastic perturbations of individual development have been referred to as developmental stability (Waddington 1957; Debat and David 2001; Klingenberg 2003). Individual and population differences in developmental stability and canalization suggest that these properties may be quite evolvable. Although selection acts on the realized variation, it is unclear how efficiently it can act on the variational potential, and the relationship between selection on variation and selection on variability is far from simple (Hansen 2006).

The direct effect of selection on variation depends on the convexity of the fitness function (Layzer 1980). Concave fitness functions (negative second derivatives) reduce variance, whereas convex fitness functions (positive second derivatives) increase variance over an episode of selection. Thus, variance is expected to decrease under stabilizing selection and increase under disruptive selection. The direct effect of linear directional selection on variance depends on the skew of the trait distribution (e.g., Bürger 1991; Hansen 1992), but with polygenic traits where near-Gaussian distributions are generated by recombination, we do not expect strong selection on variance. It has recently been shown, however, that rapid changes of additive genetic variance can occur under linear selection in the presence of directional epistasis, that is, when genes systematically modify each other in particular directions in the morphospace; positive directional epistasis leads to an increase in additive variance, whereas negative directional epistasis leads to canalization (Hansen and Wagner 2001; Carter et al. 2005; Hansen et al. 2006a). Prolonged directional selection is, however, unlikely to be common in nature, and it is unclear what general effects fluctuating directional selection may have on genetic variance components (but see Bürger 1999; Kawecki 2000; Jones et al. 2004, 2007; Draghi and Wagner 2008 for some results from specific models). The short-term response of genetic variance to stabilizing selection is also complicated and depends strongly on many different aspects of genetic architecture (e.g., Barton and Turelli 1987; Turelli and Barton 1990; Bürger 1991, 2000; Wagner et al. 1997; Hermisson et al. 2003). Over longer time scales, epistatic genetic architectures may have a tendency to evolve a degree of genetic canalization under stabilizing selection (Hermisson et al. 2003; Alvarez-Castro et al. 2009), but it is unclear whether this can have significant effects on shorter time scales or in the face of unavoidable indirect selection pressures. Similarly, theoretical analyses agree that disruptive selection may lead to an initial increase in genetic variance, and may eventually lead to bimodal distributions and lineage splitting (e.g., Bulmer 1971, 1980; Sorensen and Hill 1983; Bürger 2002; Gavrilets 2004; Spichtig and Kawecki 2004; Kopp and Hermisson 2006), but the effects of disruptive selection on genetic canalization are largely unknown. The evolution of the environmental components of variance under selection for variation is even less well understood, but Zhang and Hill (2005; Zhang 2005) found a tendency for the evolution of environmental canalization under stabilizing selection and a tendency for the evolution of environmental decanalization under fluctuating selection.

There are several problems with the idea that variational properties can be selected as adaptations. One is that selection for variational properties is most often a weak second-order effect (Proulx and Phillips 2005), susceptible to being overshadowed by indirect selection (Hansen 2010) or genetic drift (Lynch 2007). The question also remains whether variational properties are sufficiently evolvable to respond to whatever selection pressures they encounter. For example, it is generally thought that genetic canalization is not evolvable under an additive genetic architecture (e.g., Flatt 2005; but see Hansen 2003, 2010 for a proposed mechanism based on "heritable allelic effects"). With epistasis, several studies have found that a degree of genetic canalization can evolve under stabilizing selection, but that this is a relatively weak and slow process sensitive to the genetic architecture and strength of selection (Wagner et al. 1997; Hermisson et al. 2003; Alvarez-Castro et al. 2009). The most likely mechanism for the evolution of genetic canalization may in fact be as an indirect effect of selection for environmental canalization (Gavrilets and Hastings 1994; Wagner et al. 1997; de Visser et al. 2003; Rifkin et al. 2005). Empirically, much effort has been devoted to studying the evolvability of developmental stability. Most of these studies have found that fluctuating asymmetry has low additive genetic variation, but whether this translates into a similar low evolvability of developmental stability is less clear (Van Dongen and Lens 2000; Santos 2002; Fuller and Houle 2003; Pélabon et al. 2004a; Leamy et al. 2005; Leamy and Klingenberg 2005).

Although the theoretical results paint a complex picture of the evolution of variational properties under different selective regimes, we can identify some general hypotheses that can be tested against data by answering the following questions: (1) Can variational properties be changed within the time scale of a selection experiment, and if these changes occur, are they predictable from the expected effects of selection on variation? (2) Alternatively, are canalization and developmental stability generally optimized under natural selection, so that we may expect decanalization and increased developmental noise under most artificial-selection regimes? (3) Are the variational properties of genetic, environmental, and individual developmental components concordant? Indeed, although developmental stability and canalization should be similarly affected by selection on variation, it is unknown whether the underlying bases of these

variational properties are distinct. Although several authors have proposed that canalization and developmental stability may be influenced by common buffering mechanisms (Klingenberg 2003; Flatt 2005), empirical data are highly inconsistent; some studies support the idea of common regulatory mechanisms (Clarke 1998; Klingenberg and McIntyre 1998; Hallgrímsson et al. 2002; Willmore et al. 2005; Breuker et al. 2006), but others reject this hypothesis (Debat et al. 2000; Hoffmann and Woods 2001; Réale and Roff 2003; Pélabon et al. 2004b; Debat et al. 2006, 2009).

To test these hypotheses, we analyzed data from artificial-selection experiments that exposed *Drosophila melanogaster* populations to more than 20 generations of stabilizing, fluctuating, and disruptive selection on individual differences in a wing shape index. Selection was performed on an index derived from two traits corresponding to the relative position of wing veins. We compared the effects of the different selection regimes on the within-individual variance (phenotypic expression of developmental stability) estimated by the level of fluctuating asymmetry and on the among-individual variance (combination of standing genetic variation and genetic and environmental canalization) for the two traits composing the selection index and for wing size, which was not directly selected. We also analyzed the effects of the different selection regimes on directional asymmetry. Although directional asymmetry in shape and size are widespread in insect wings (Pélabon and Hansen 2008), its short-term evolvability is unknown. Indeed, despite the absence of response to selection on directional asymmetry (Maynard-Smith and Sondhi 1960; Coyne 1987; Carter et al. 2009), we have previously found that directional asymmetry can respond indirectly to selection on wing shape (Pélabon et al. 2006), and Rego et al. (2006) demonstrated a considerable potential for genetic variation in directional

asymmetry in interspecific hybrids. We therefore tested if the selection regimes applied here had similar effects on directional asymmetry in wing size and shape.

Material and Methods
SELECTION PROCEDURE

We used two different base populations for the selection experiments. The IV population descended from about 200 flies collected by P. T. Ives in Amherst, Massachusetts in 1975. These flies have been maintained by B. Charlesworth (1976–1992) and D. Houle (1992 onwards) since that time in laboratory conditions under a 12:12 L:D cycle at 25°C in bottles with transfers every 14 days (see Houle and Rowe 2003 for more details about this line). The LHM population descends from 400 flies collected by L. Harshman in central California in 1991. In 1995, 2000 of these flies were used to found a subpopulation maintained by W. R. Rice until it was obtained by the Houle lab shortly before initiating the selection experiments in 2004. During the selection experiment flies were reared at 25°C in plastic shell vials (95 mm height, 25 mm diameter) containing corn-meal, sucrose, dead-yeast medium without the addition of live yeast.

To perform selection on the shape of the wing, we measured wings from live flies using an automated image-analysis system (WINGMACHINE, Houle et al. 2003). Each wing 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). A digital image of the wing was then recorded using a macroscope. Cubic B-splines (Lu and Milios 1994) were fitted to the vein structure distal to a line defined by user-supplied landmarks (dashed line Fig. 1) (Houle et al. 2003). For the analysis of the asymmetry
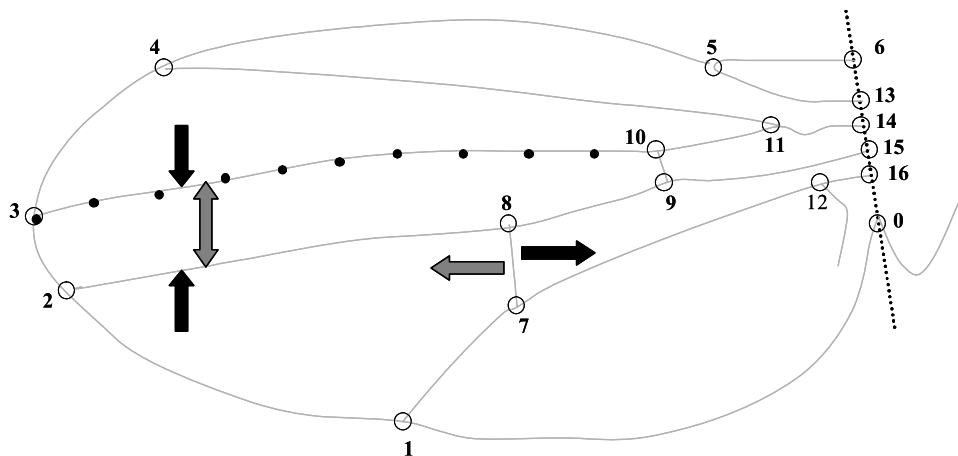

Figure 1. Representation of the wing of *Drosophila melanogaster* with the reference numbers of the different landmarks used in this analysis (landmarks 0 and 13–16 are not used in the analysis). The effects of the selection on the position of the veins are represented by the arrows (decreasing index: black arrows; increasing index: grey arrows). Black dots along the vein III represent the evenly distributed reference points where the distance between the vein III and IV is calculated to estimate the first trait composing the selection index (see text for details).

patterns, we imaged both left and right wings, reversed the right image, and used the same B-spline model to fit both images.

Selection was performed on an index derived from two traits defined from the fitted spline models. For logistic reason, only the left wing was photographed and measured during the selection process. Therefore, selection was performed on measurements taken from the left wing only. The first trait, I_1 measured the distance between veins III and IV, defined as

$$I_1 = \frac{\text{average distance between veins III and IV}}{\sqrt{\text{total wing area}}}.$$

The average distance was calculated by taking 10 evenly spaced points along vein III distal to the anterior cross-vein (between landmark 3 and 10, shown as black dots in Fig. 1). We calculated the distances from each of these 10 points to the closest point on vein IV, averaged these 10 distances, and standardized for wing size by dividing by the square root of the area enclosed by the outer spline function. The second trait, I_2, measured the relative position of the posterior cross-vein, defined as

$$I_2 = (\mathrm{d}[15, 8]/\mathrm{d}[15, 2] + \mathrm{d}[16, 7]/\mathrm{d}[16, 1])/2,$$

where $\mathrm{d}[a, b]$ is the linear distance between the landmarks a and b. The standard deviation of trait I_1 was initially 2.6 times smaller than that of I_2. To perform selection of equal strength on the two traits, the selection index was a weighted sum of the two traits $I = 2.6 \times I_1 + I_2$. By the time of the measurements, the average ratio (±standard error) between the standard deviation of the two traits in each selection treatment was: Control: 2.65 ± 0.18; Disruptive: 2.54 ± 0.37; Fluctuating: 2.42 ± 0.23; Stabilizing: 2.53 ± 0.21. Therefore, despite changes in the phenotypic variance of the two traits (see results), the strength of selection applied on both traits was approximately constant during the course of the experiment. The selection lines were started early spring 2004.

In each selected line, 100 virgin flies from each gender were measured at each generation and 25 from each gender selected as parents of the next generation. There were five selection regimes plus a control, each replicated twice in each of the two base populations. The selection regimes were up, down, stabilizing, disruptive, and fluctuating selection. In the stabilizing-selection lines, we sorted the flies within each gender by their selection-index score and chose 25 flies with consecutive scores such that their average score was closest to the average value at the start of the experiment. In the disruptive-selection lines, we chose 25 flies of each gender from the extreme ends of the distribution in such a ratio that their average score was closest to the starting average value (e.g., the top 10 and lowest 15 or the top 12 and lowest 13). Fluctuating selection was achieved by computing the mean of the selection index for the 200 flies measured. If this value was lower than the starting value, directional selection to increase the index value (up selection) was performed, whereas directional selection

to decrease the index value (down selection) was performed when the average score was above the starting value. As a result, the direction of selection changed nearly every generation. For the up (and down) line the 25 males and females with the highest (or lowest) within-gender scores were selected for mating. For the control lines, 25 individuals of each gender were haphazardly chosen.

For mating, we placed five selected individuals of each gender in five vials. In each treatment, flies were randomly assigned to a mating vial. These parental flies were transferred to new vials after approximately 24 h and the individuals for the next generation were collected as virgins approximately—eight to nine days later. The effects of the two directional-selection treatments on fluctuating and directional asymmetry have been presented elsewhere (Pélabon et al. 2006); we report here the results for the other treatments.

MEASUREMENTS AND ANALYSIS

At generation 21 for IV_2 and LHM_2 and generation 22 for IV_1 and LHM_1 (indices refer to the replicate number), we imaged both left and right wings for about 100 female flies in each line. In approximately half of these flies we imaged and measured both wings a second time to estimate measurement error. Data were registered and size-corrected using the generalized least-squares Procrustes superimposition (Rohlf and Slice 1990). We analyzed the effect of the different selection regimes on the variational properties of three traits. The first trait was the average distance between landmarks 2 and 3, and 9 and 10 (Fig. 1). This mimics, but does not replicate, index I_1, because it uses only the size-corrected distances at the ends of the selected region, rather than all along it. The second trait corresponded to I_2, defined above. Finally, we analyzed wing size measured by the centroid size (the square root of the sum of squared distances from each landmark to the centroid) based on landmarks 1–12. Wing size was not a direct target of selection because both traits composing the selection index were corrected for wing size.

The full model fit followed Palmer and Strobeck (1986) and Palmer (1994) in including Side as a fixed effect to test for directional asymmetry. In this model, the Individual (random effect) × Side interaction term estimates the variance due to fluctuating asymmetry, whereas the error term estimates measurement error for replicated measurements. As we also had Treatments, Populations and Replicated selection lines nested within Treatments and Populations, the full model included: Treatment, Side and Side × Treatment interaction as fixed effects, and Population, Population × Treatment, Replicate within Population and Treatment, Individual within Replicate, Individual × Side interaction as random factors. Models were fitted in Proc Mixed in SAS 9.1 (SAS Institute, Cary, NC). To stabilize estimates of variance components in Proc Mixed, all data were multiplied by 100.

Ideally, a test for differences in trait variances among treatments would be performed directly in Proc Mixed in SAS using the group = treat option. Unfortunately, this was not possible due to limited computation power. Therefore, we fitted separate models to each treatment, and then compared the AIC values of these unconstrained models with the AIC values of similar models in which the variance parameters were constrained to equal the estimates from the full analyses. We summed the log-likelihood values for the constrained and unconstrained models across treatments, and calculated the AIC (AIC = −2 log(likelihood) + 2p, where p is the number of variance components following SAS Mixed procedure).

Finally, to directly test for differences in variances, we estimated the best unbiased linear predictors (BLUPs) for each level of each random effect from the full model. To check the measurement error, we calculated the residuals from the full model for the subset of individuals that were measured twice. We then performed Levene tests, one-way analyses of variance (ANOVAs) on the absolute value of these BLUPs and residuals (Palmer and Strobeck 1992) with Treatment as factor.

Fluctuating-asymmetry analyses are particularly sensitive to outliers. We used a multistep procedure to remove potential outliers. First, we noted apparent outliers for centroid size and each landmark coordinate after Procrustes alignment. The original images of wings with unusual phenotype for any of these traits were then examined for errors, such as the wing being slightly folded or damaged, or from a badly fitted B-spline. Those with apparent errors were excluded from further analysis.

Second, for those flies imaged and measured twice, we fitted a full mixed-model analysis of individual wings for the two shape traits and centroid size implemented in Proc GLM. The resulting residuals reflect the differences between wings within replicate measurements. We performed Grubb's test for outliers with $P = 0.01$, and $N = 2 \times 450$, the approximate sample size of twice measured wings within each treatment. When a residual was tested as an outlier, the offending replicate was excluded from further analysis (both wings for all traits). This resulted in wings with residuals more than 4.3 SD from the mean being identified as outliers. Of a total of 880 flies where both wings were measured twice, 32 pairs of wings were dropped. A final round of outlier removal was done at the level of individual flies. We averaged the replicate measurements of the same wings, and calculated the value of left–right wing for each trait. Grubb's test was then applied within each treatment, with the critical values calculated with $P = 0.01$ and $N = 450$. Individuals with wing measurements > 4.05 SD from the mean were discarded. Twelve individuals were dropped as outliers at this step. The final dataset contained 860 individuals where each wing was measured twice and 794 individuals where both wings were measured once.

In three of four cases, the measurement variances for the two traits under selection were the highest in the disruptive-selection lines (Appendix S1). Although we did not observe obvious problems in the fit of the B-spline, it is possible that the particular shape of some of the wings in the disruptive-selection lines affected this fit, and therefore increased the measurement variance. Although this variation in measurement error does not affect our results because estimates of fluctuating asymmetry were corrected for measurement variance, it underlines the importance of estimating measurement variance in each selection line with a sufficiently large sample size of repeated measures.

Following Hansen et al. (2006b), we calculated the relative developmental imprecision as the part of phenotypic variation due to developmental instability, i.e., the part of phenotypic variation due to within-individual variation measured by the fluctuating asymmetry. The developmental imprecision was obtained by dividing the Individuals × Side variance component by the total among-individual variance component (including the within-individual variance, in Tables 2–4).

Results
TRAIT MEANS AND AMONG-INDIVIDUAL PHENOTYPIC VARIANCE

As intended, neither the average wing size nor the mean of the two shape characters were significantly affected by the different selection treatments (Table 1; Appendix S1). Analyses of each of the three traits showed that Selection treatment, Population, and Population × Selection treatment means were never statistically significantly different from zero when all effects were in the model ($P > 0.28$ in all cases). Given the lack of Population and Population × Selection treatment effects on the traits mean, we chose to treat Population as a fixed effect and to drop the Population × Selection treatment effect from further analyses.

The among-individual variance for both traits under selection was strongly affected by the selection treatment (Fig. 2A, B, Table 2 and 3). As expected, lines under disruptive selection displayed the highest among-individual variance, whereas lines under fluctuating and stabilizing selection generally displayed levels of among-individual variance lower than the control lines. Among-individual variance in centroid size, however, tended to decrease under stabilizing and fluctuating selection (significantly so in the latter), but did not increase under disruptive selection (Fig. 2C, Table 4).

In addition to the marked effects on the phenotypic variance of the two selected traits, disruptive selection also affected the development of the wing. In a few cases, we observed individuals with anomalous wing veins (Fig. 3). Because these anomalies prevented us from properly fitting the cubic B-splines and

Table 1. Results of mixed-effects model analyses of variance of the two shape traits and the centroid size for the full dataset. The units for trait 1 are the average distances between veins III and IV in centroid size units×100; the unit for trait 2 is the proportional position of the distal cross-vein×100.

Source	Trait 1		Trait 2		Centroid size	
	Variance±SE	P	Variance±SE	P	Variance±SE	P
Treatment		0.90		0.86		0.79
Side		0.0001		0.0001		0.0001
Treatment × Side		0.014		0.37		0.042
Population		0.58		0.043		0.79
Replicate in Population × Treatment	12.612±5.485	0.011	39.218±17.528	0.013	17.269±7.626	0.012
Individual in Replicate	22.773±0.900	<0.0001	168.850±6.662	<0.0001	63.517±2.239	<0.0001
Individual × Side	3.922±0.201	<0.0001	37.674±1.434	<0.0001	0.975±0.0388	<0.0001
Residual	2.269±0.078	<0.0001	4.573±0.156	<0.0001	0.180±0.0062	<0.0001
$-2 \log(\Lambda)$	26,265.7		34,058.4		20,753.4	
AIC	26,273.7		34,066.4		20,761.4	

accurately estimate the position of the landmarks, these flies were discarded during the selection phase and unfortunately we do not have estimates of the frequency with which these anomalies were encountered.

FLUCTUATING ASYMMETRY AND DEVELOPMENTAL PRECISION

The shape characters displayed an average fluctuating asymmetry in the control lines of 2.20% ± 0.20% and 1.17% ± 0.10% of the trait size, for the trait 1 and 2, respectively. In comparison, centroid size displayed an average level of fluctuating asymmetry of 0.47% ± 0.04% (Appendix S1). Contrary to the effects on among-individual variance, all selection treatments provoked an increase in fluctuating asymmetry in both selected traits and in wing size (Fig 4; Table 2–4). These increases were sometimes substantial, with an average increase in the within-individual variance in the disruptive-selection lines of 21%, but remained limited compared to the 412% and 530% increase in among-individual variance under disruptive selection in trait 1 and 2, respectively.

Changes in within- and among-individual variance among selection treatments were inconsistent, leading to marked variation in the relative developmental imprecision of the two selected traits (Fig. 5A, B). Because the effect of disruptive selection on these traits was more pronounced on phenotypic variation than on fluctuating asymmetry, the relative developmental imprecision strongly decreased in the disruptive-selection lines despite an increase in fluctuating asymmetry. Because phenotypic variation in centroid size was not affected by the different selection treatments, the relative developmental imprecision in this trait primarily reflected changes in fluctuating asymmetry (Fig. 5C).

DIRECTIONAL ASYMMETRY

As reported in earlier studies on asymmetry in *Drosophila* wings (see Pélabon and Hansen 2008 for a review), we observed statistically significant directional asymmetry (Side effect in Tables 2–4) of small magnitude in both wing size (centroid size) and wing shape (traits 1 and 2). In the control lines, the distance between veins III and IV was on average 0.64% ± 0.17% larger in the left wing, the position of the posterior cross-vein was 0.18% ± 0.05% more distal on the right wing, and the left wing was 0.23% ± 0.04% larger than the right one.

Directional asymmetries in trait 1 and centroid size were affected by the selection treatments (Selection treatment × Side effect in Table 1). The pattern of these effects, however, was idiosyncratic. Directional asymmetry increased in all selected lines for trait 1 (Fig. 6A), whereas disruptive and fluctuating selection tended to decrease directional asymmetry in wing size (Fig. 6C). Directional asymmetry in the position of the posterior cross-vein (trait 2) did not show large or statistically significant effects of any selection treatment (Fig. 6B, Table 1).

Discussion

Our results from slightly more than 20 generations of disruptive, fluctuating, or stabilizing selection on the *Drosophila* wing conform for the most part to the traditional expectations for the evolution of among-individual variation. Phenotypic variance strongly increased under disruptive selection but decreased under fluctuating and stabilizing selection relative to the controls. The increases in phenotypic variance in the disruptive selection lines were also accompanied by the appearance of individuals with highly deviant phenotypes. Phenotypic variance in wing size, however, was
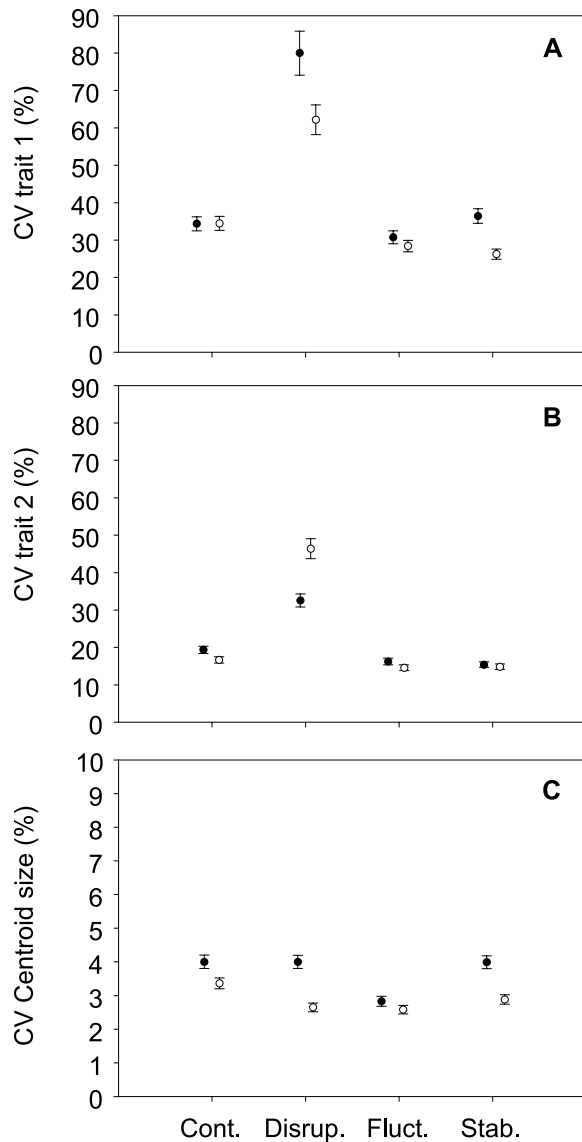
Figure 2. Coefficients of phenotypic variation (±SE) of the trait 1 (A), trait 2 (B), and centroid size (C) for the two populations (LHM: open symbols; IV: black symbols) in the different selection treatments (replicates combined).

unaffected or modestly decreased in all selected lines. Although this was not entirely surprising considering that wing size was not under selection, this also supports the hypothesis that the genetic controls of shape and size in the *Drosophila* wing are independent (Gilchrist and Partridge 2001, but see Breuker et al. 2006). Effects of the different selection treatments on the within-individual variance were, however, largely inconsistent with the expectations, because fluctuating asymmetry increased in all selection treatments for wing size and both shape characters.

Directional asymmetry was affected by the different selection treatments, but in a rather unpredictable manner. Directional asymmetry in size and shape is widespread in insect wings

(Pélabon and Hansen 2008), and has even been considered as adaptive (Van Valen 1962), but all direct attempts at detecting genetic variance in this trait have failed (Maynard Smith and Sondhi 1960; Tuinstra et al. 1990; Carter et al. 2009), indicating a lack of evolutionary potential. This apparent lack of genetic variation is, however, contradicted by the observations of changes in directional asymmetry in response to selection on wing shape (Pélabon et al. 2006; this study), by the increase in the level of directional asymmetry in wing size observed in hybrids between two *Drosophila* species (Rego et al. 2006), and the variation in direction and magnitude of directional asymmetry in wing size observed across, and even sometimes within insect species (Pélabon and Hansen 2008).

Although these results appear contradictory, Pélabon and Hansen (2008) found that directional asymmetry in wing size was expressed with extremely poor developmental precision, which implies that a genetic signal for directional asymmetry could be swamped by developmental noise. Pélabon and Hansen (2008) therefore suggested that this might explain the repeated failure of directional asymmetry to respond to direct selection, because the lack of developmental precision would make selection extremely inefficient despite possible genetic variation in directional asymmetry. It is still possible, however, that directional asymmetry can evolve via a correlated response to selection if its genetic variation is correlated with genetic variation in other traits. Such a correlated response to selection could explain the idiosyncratic changes in directional asymmetry we and other have observed.

Using recombining populations increases the realism of the study but limits our ability to infer the relative contribution of the different processes that affect the among-individual phenotypic variation and therefore our capacity to interpret the results in terms of changes in canalization. Indeed, changes in the among-individual variance can result from either changes in genetic and/or environmental canalization, changes in allele frequency due to selection or drift, or a combination of these effects. These difficulties, however, do not extend to the effect of selection on developmental stability because variation in the level of fluctuating asymmetry in a constant environment is expected to be a direct reflection of changes in developmental stability. Keeping the above-mentioned limitations in mind, we now discuss our results in relation to the questions posed in the introduction about the evolution of variational properties.

(1) Can variational properties be changed within the time scale of a selection experiment, and if these changes occur, are they predictable from the effects of selection on variation?

Disruptive selection is expected to maintain or even increase genetic variation by equalizing allele frequency at polymorphic loci (Bulmer 1971; Spichtig and Kawecki 2004). Linkage disequilibrium may further increase genetic variance creating a bimodal distribution of the phenotypes. This could occur under assortative

Table 2. Results of mixed-effects model analyses of variance of trait 1 within treatments. The unit of measurement is the average distances between veins III and IV (see text for full explanation) in centroid size units × 100. Models with a constant replicate-in-population variance over treatments fit best, so these variances were constrained to the estimate from the full model shown in Table 1. Models that allowed the other three random effects to vary among treatments fit better than the model with constant variance across treatments (total AIC difference: 687). The analysis based on the best unbiased linear predictor (BLUPs) confirmed that there were no significant differences in variance for the replicate within population and treatment term. The effect of selection treatments on the fluctuating asymmetry (Individual × Side variance) was significant; Levene test on the BLUPs: *P*<0.0001.

Source	Control		Disruptive		Fluctuating		Stabilizing	
	Variance±SE	P	Variance±SE	P	Variance±SE	P	Variance±SE	P
Side		<0.0001		<0.0001		<0.0001		<0.0001
Population		0.97		0.63		0.96		0.86
Replicate in Population	12.61		12.61		12.61		12.61	
Individual in Replicate	11.99±1.00	<0.0001	61.02±4.52	<0.0001	7.53±0.74	<0.0001	9.35±0.82	<0.0001
Individual × Side	3.16±0.31	<0.0001	5.44±0.60	<0.0001	3.57±0.35	<0.0001	3.38±0.34	<0.0001
Residual	1.79±0.12	<0.0001	3.77±0.27	<0.0001	1.70±0.12	<0.0001	1.92±0.13	<0.0001
−2 log(Λ)	6218.2		7330.9		5693.8		6311.8	
AIC	6226.2		7338.9		5701.8		6319.8	

mating, but also under random mating, because mating between two up- or down-selected individuals would be more likely to produce offspring with an extreme phenotype that will fall in the selected fraction in the next generation than mating between one up- and one down-selected individual, which would tend to produce intermediate offspring. Alternatively, phenotypic variation could increase via either genetic or environmental decanalization. On the one hand, the low kurtosis and the signs of bimodality observed in the distributions of the traits in the disruptive-selection lines (Appendices S1 and S2) tend to confirm the former hypothesis because neither genetic nor environmental decanalization is expected to affect the kurtosis of the trait distribution. On the other hand, the consistency of the phenotype of the anomalous wings

observed in the disruptive-selection lines provides some evidence in favor of genetic decanalization. Because we discarded these anomalous individuals from the experiment and our analyses, it remains uncertain whether and how much of the increase in phenotypic variance resulted from genetic decanalization.

Stabilizing selection can reduce variation in several ways: removal of alleles with large effect, genetic or environmental canalization, or an increased proportion of fixed alleles via drift, because correlations in reproductive success across generations decrease the effective population size. The fact that phenotypic variance in wing size decreased regardless of the selection regime is consistent with a decrease in effective population size as the causal factor, but this decrease in within-line variance was not

Table 3. Results of mixed-effects model analyses of variance of trait 2 within treatments. The unit of measurement is the proportional position of the distal cross-vein (see text) × 100. Models with a constant replicate-in-population variance over treatments fit best, so these variances were constrained to the estimate from the full model shown in Table 1. Models that allowed the other three random effects to vary among treatments fit better than the model with constant variance across treatments (total AIC difference=775.1). The effect of selection treatments on the fluctuating asymmetry (Individual × Side variance) was significant; Levene test on the BLUPs: *P*<0.0001.

Source	Control		Disruptive		Fluctuating		Stabilizing	
	Variance±SE	P	Variance±SE	P	Variance±SE	P	Variance±SE	P
Side		0.011		0.050		0.0072		0.61
Population		0.75		0.42		0.09		0.30
Replicate in Population	39.2		39.2		39.2		39.2	
Individual in Replicate	79.11±6.80	<0.0001	498.45±36.37	<0.0001	43.06±4.81	<0.0001	44.94±4.46	<0.0001
Individual × Side	30.67±2.39	<0.0001	50.22±3.74	<0.0001	37.42±2.93	<0.0001	32.31±2.43	<0.0001
Residual	4.60±0.31	<0.0001	5.14±0.36	<0.0001	4.21±0.29	<0.0001	4.37±0.29	<0.0001
−2 log(Λ)	8274.5		9127.0		7611.8		8246.0	
AIC	8282.5		9135.0		7619.8		8254.0	

Table 4. Results of mixed-effects model analyses of variance of centroid size (mm) × 100 within treatments. Models with a constant replicate-in-population variance over treatments fit best, so these variances were constrained to the estimate from the full model shown in Table 1. Models that allowed the other three random effects to vary among treatments fit better than the model with constant variance across treatments (total AIC difference=60.9). The effect of selection treatments on the fluctuating asymmetry (Individual × Side variance) was significant; Levene test on the BLUPs: *P*<0.0034.

Source	Control		Disruptive		Fluctuating		Stabilizing	
	Variance±SE	*P*	Variance±SE	*P*	Variance±SE	*P*	Variance±SE	*P*
Side		<0.0001		0.0010		0.0011		<0.0001
Population		0.49		0.94		0.90		0.77
Replicate in Population	17.27		17.27		17.27		17.27	
Individual in Replicate	76.714±5.378	<0.0001	64.564±4.506	<0.0001	41.386±3.044	<0.0001	69.525±4.798	<0.0001
Individual × Side	0.818±0.066	<0.0001	1.144±0.090	<0.0001	1.089±0.090	<0.0001	0.860±0.067	<0.0001
Residual	0.168±0.011	<0.0001	0.196±0.014	<0.0001	0.207±0.014	<0.0001	0.153±0.010	<0.0001
−2 log(Λ)	5217.4		5347.7		4818.9		5284.5	
AIC	5225.4		5355.7		4826.9		5292.5	

accompanied by an increase in the among-line variance as expected under drift. Therefore, a decrease in genetic variance via selection and/or an increase in genetic and environmental canalization remain the most likely mechanisms that affected phenotypic variation under stabilizing selection. Scharloo et al. (1967) also observed that the decrease in phenotypic variation under stabilizing selection was partly caused by the decrease in the genotype sensitivity to environmental variation (i.e., increased environmental canalization).

Our fluctuating-selection treatment applied truncated directional selection that changed direction nearly every generation. Over the course of the experiment this regime could have resulted in net stabilizing selection. The effects of such a selection treatment on random environmental variation (i.e., variation unrelated to environmental cues; Bull 1987) are less predictable, however, because intermediate genotypes with a high phenotypic variance due to low environmental canalization or developmental stability could have larger geometric mean fitness than more canalized
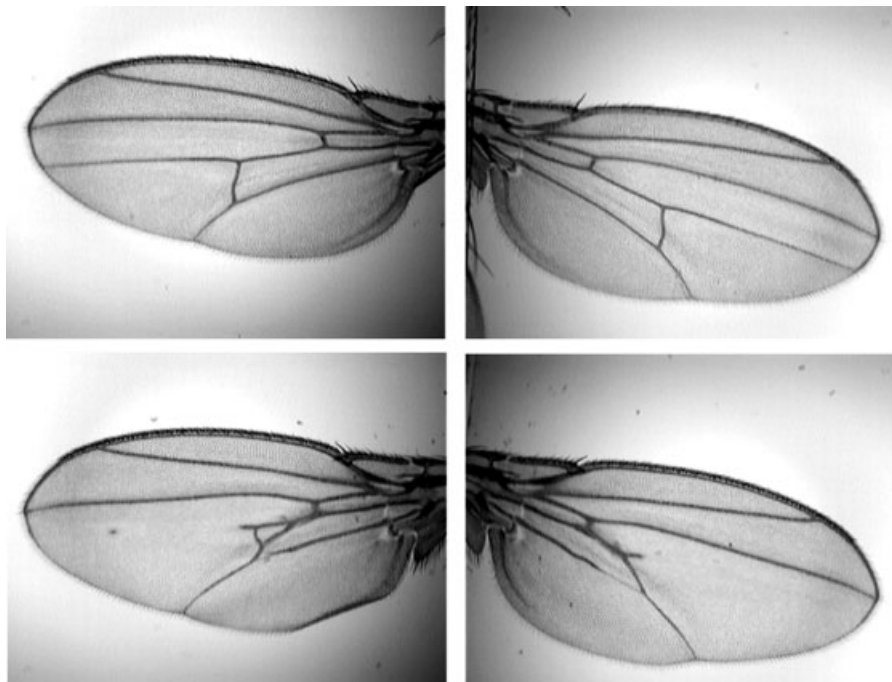

Figure 3. Example of the abnormal development of the *Drosophila* wing observed in the disruptive selection lines (upper panel: normal wings; lower panel: wings with anomalous development).
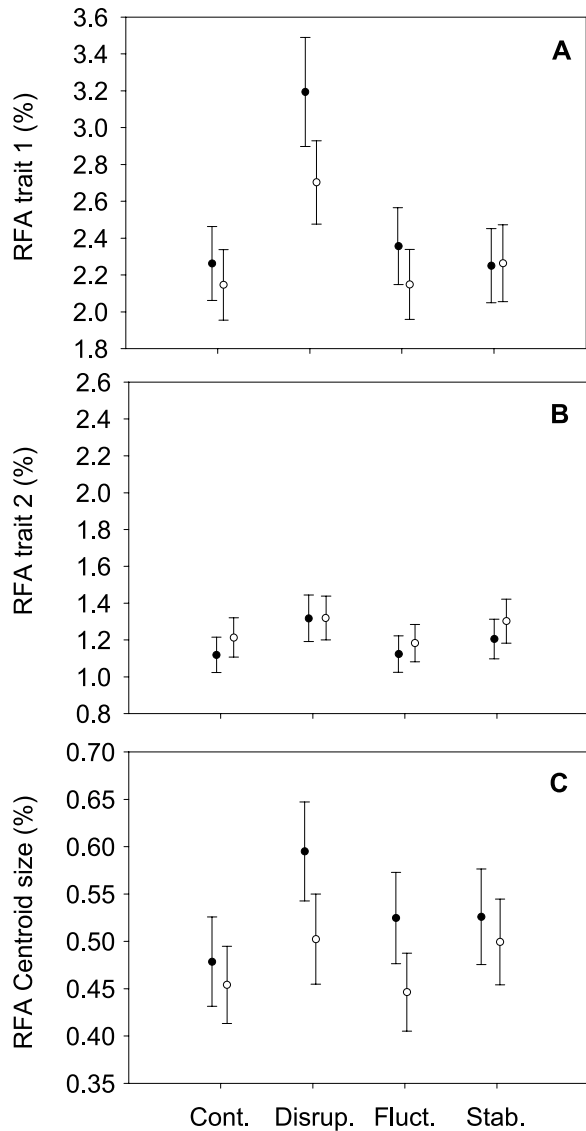
Figure 4. Mean (±SE) relative fluctuating asymmetry (RFA, size standardized fluctuating asymmetry) in the trait 1 (A), trait 2 (B), and in centroid size (C) for the two populations (LHM: open symbols; IV: black symbols) in the different selection treatments. The size standardized FA was obtained by dividing the average fluctuating asymmetry (corrected for the directional asymmetry) by the average trait size (replicates combined). RFAs are given in percentage of the mean trait size. Therefore, a mean RFA of 2.2% in the trait 1 indicates that the average asymmetry between the left and the right wings in the distance between veins III and IV equals 2.2% of the average distance between the two veins.

Figure 5. Developmental imprecision (±SE) of the trait 1 (A), trait 2 (B), and centroid size (C) for the two populations (LHM: open symbols; IV: black symbols) in the different selection treatments. The developmental imprecision is expressed as the percentage of phenotypic variance due to fluctuating asymmetry. It is obtained by dividing the total among-individual variance component (including the within-individual variance) by the individual × Side variance component (replicates combined).

genotypes (Bull 1987; Simons and Johnston 2006). Following this scenario, the decrease in phenotypic variance resulting from the loss of extreme genotypes could be balanced by the increasing frequency of genotypes with intermediate means, but increased variance. Our results support this hypothesis because we observed a simultaneous decrease in phenotypic variance and increase in
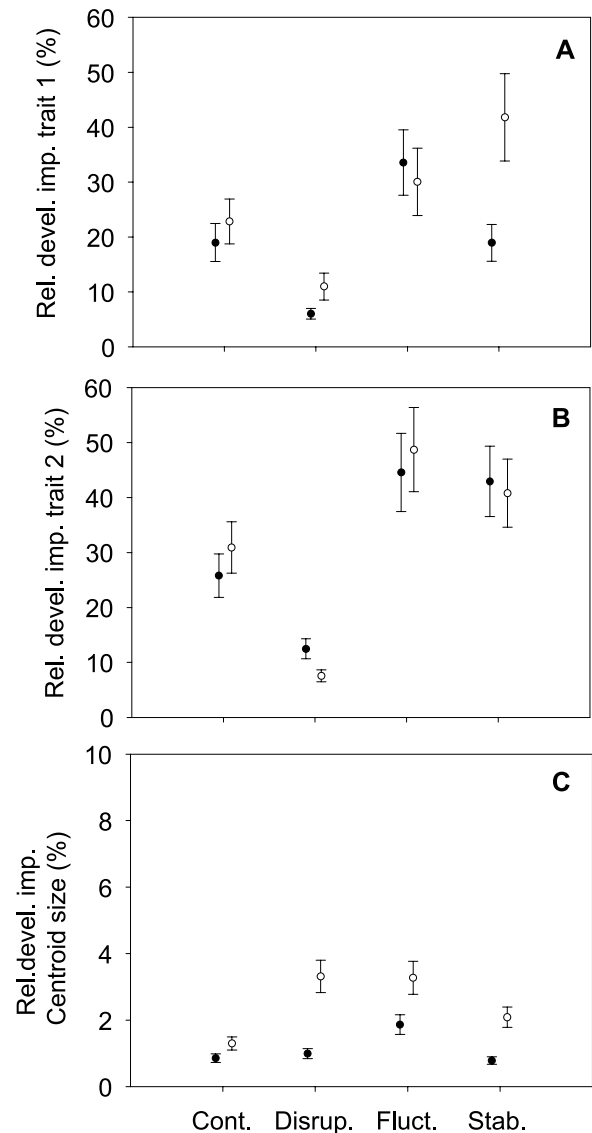
developmental instability. Nevertheless, despite the possible decrease in environmental canalization under fluctuating selection, this treatment appeared more efficient at decreasing phenotypic variation than stabilizing selection, suggesting that rapid fluctuating selection as applied here was particularly efficient at removing alleles with larger effects.

Developmental stability was clearly affected by selection on variation. Fluctuating asymmetry in all traits increased under disruptive selection, suggesting that this selection treatment
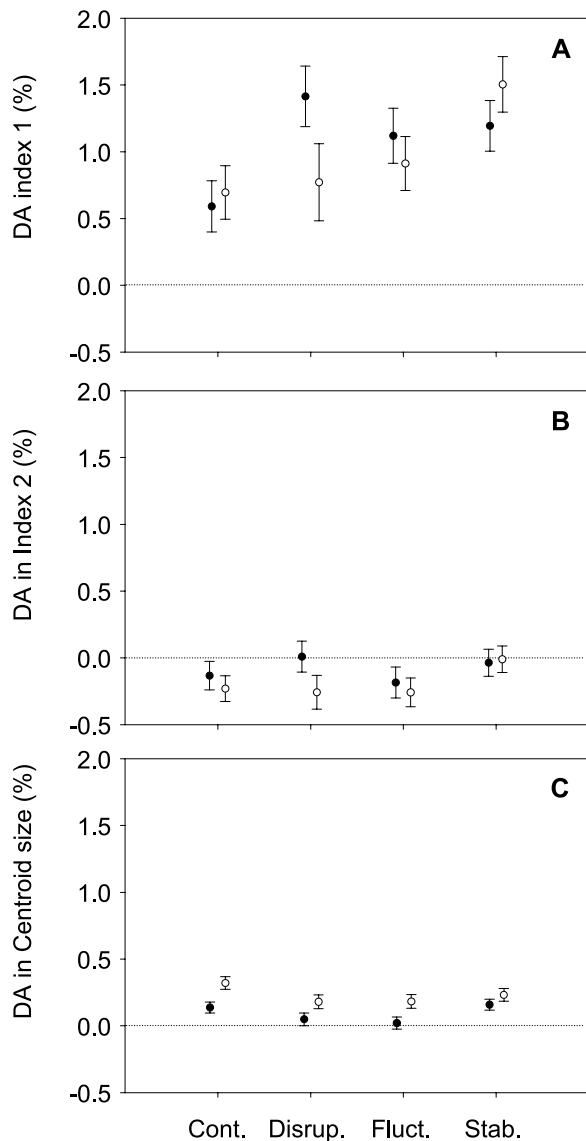
Figure 6. Mean (±SE) directional asymmetry expressed in percentage of trait size in trait 1 (A), trait 2 (B), and in centroid size (C) for the two populations (LHM: open symbols; IV: black symbols) in the different selection treatments (replicates combined).

negatively affects developmental stability, as expected from some models. This result seems to confirm earlier studies by Scharloo et al. (1967) and Thoday (1959) where disruptive selection provoked an increase in both phenotypic variation and fluctuating asymmetry. However, the increase in fluctuating asymmetry in the disruptive-selection lines is not necessarily an adaptive response of developmental stability to selection on variation. Indeed, fluctuating asymmetry in wing size also increased although no selection was applied on this character. Furthermore, stabilizing selection provoked a weak but consistent increase in fluctuating asymmetry. This last result is in sharp contrast with the general expectation that stabilizing selection should decrease any

sources of phenotypic variation. It also contrasts with the results from Scharloo et al. (1967). However, selection in Scharloo's experiment started immediately after the introduction of a mutant allele into the reference genetic background. Although this was justified to prevent natural selection on the mutant character, introduction of the mutant allele may have disrupted developmental stability. The subsequent increase in developmental stability may have resulted from the reorganization of the genetic architecture around the new allele, as observed in *Lucilia cuprina* (Clarke and McKenzie 1987), and not as a response of developmental stability to selection on variation.

(2) Are canalization and developmental stability generally optimized under natural selection, so that we may expect decanalization and increased developmental noise under most artificial-selection regimes?

The nonadaptive changes in fluctuating asymmetry suggest that developmental instability may be effectively optimized in the control lines and that any changes in the genetic background resulting from whatever selection regimes would lead to a decrease in developmental stability. This hypothesis finds further support in the asymmetrical responses that A. J. R. Carter and D. Houle (unpubl. ms.) obtained when selecting flies from the LHM population for an increase and a decrease in fluctuating asymmetry in wing shape. Although lines selected for an increase in fluctuating asymmetry showed a consistent response during the 42 generations of selection, only one of the two lines selected for a decrease in fluctuating asymmetry showed a significant response to selection, this response being weaker than the response in the lines selected for an increase fluctuating asymmetry. Alternatively, the general increase in fluctuating asymmetry under selection could be explained by a negative effect of inbreeding on developmental stability (Mitton and Grant 1984) if selected lines were more homozygous than the control lines. This hypothesis is unlikely, however, because the association between developmental stability and inbreeding is generally weak even in studies where the level of inbreeding was high (Vøllestad et al. 1999), and our selected lines were maintained with the rather large population size.

Developmental stability was most affected by disruptive selection in our study. Furthermore, both Thoday (1959) and Scharloo et al. (1967) found that the negative effect of disruptive selection on developmental stability depended on the occurrence of disassortative mating (mating between opposite extremes). These observations together with the often observed decrease in developmental stability in interpopulation or interspecific hybrids (Alibert and Auffray 2003), suggest that interactions among-loci, that is, epistasis, may be the primary source of genetic variance in developmental stability, as suggested by Leamy et al. (2005).

(3) Are the variational properties of genetic, environmental, and individual developmental components concordant?

Changes in among- and within-individual variance were only congruent in the disruptive-selection lines for the selected characters, although the increase in fluctuating asymmetry was much smaller than the increase in phenotypic variance. Under stabilizing selection, where all sources of phenotypic variation should be minimized, fluctuating asymmetry increased. Furthermore, disruptive selection on the shape character did not affect, or slightly decreased the among-individual variance in wing size but increased fluctuating asymmetry. To the extent that changes in among-individual variation reflect, at least partly, changes in canalization, these results support the hypothesis that different processes affect canalization and developmental stability (Debat et al. 2000, 2006, 2009; Hoffmann and Woods 2001; Réale and Roff 2003; Pélabon et al. 2004b).

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Means and variance components for the three traits for each population in each selection treatment.
Appendix S2. Distribution of the phenotypic values for the traits under selection.

Supporting Information may be found in the online version of this article.

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