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Consequences of inter-population crosses on developmental stability and canalization of floral traits in *Dalechampia scandens* (Euphorbiaceae)

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phenotypic variance.

Abstract

Congruence between changes in phenotypic variance and developmental noise in inter-population hybrids was analysed to test whether environmental canalization and developmental stability were controlled by common genetic mechanisms. Developmental stability assessed by the level of fluctuating asymmetry (FA), and canalization by the within- and among-individual variance, were measured on several floral traits of *Dalechampia scandens* (Euphorbiaceae). Hybridization affected canalization. Both within- and among-individual phenotypic variance decreased in hybrids from populations of intermediate genetic distance, and strongly increased in hybrids from genetically distant populations. Mean-trait FA differed among cross-types, but hybrids were not consistently more or less asymmetric than parental lines across traits. We found no congruence between changes in FA and changes in phenotypic variance. These results suggest that developmental stability (measured by FA) and canalization are independently controlled. This study also confirms the weak relationship between FA and the breakdown of coadapted gene complexes following inter-population hybridization.

Introduction

Canalization describes the ability of the genome to suppress phenotypic variation due to genetic or environmental disturbances (Waddington, 1957; Wagner *et al.*, 1997). Developmental stability is the ability of an organism to buffer nonadaptive phenotypic variation resulting from micro-environmental disturbances during development (developmental noise; Waddington, 1957; Debat & David, 2001). Because not only the population mean but also the precision of the trait's development should be optimized by selection (Armbruster *et al.*, 2004), both canalization and developmental stability should be under selective pressures. However, the mechanisms that buffer development against genetic and environmental variation remain poorly understood (Rutherford, 2000). Several authors have argued that environmental canalization and developmental stability are controlled by similar genetic mechanisms, because both affect the sensitivity to environmental variation (Clarke, 1998; Klingenberg & McIntyre, 1998; but see Waddington, 1957; Debat *et al.*, 2000; Hoffmann & Woods, 2001).

Canalization and developmental stability are variational properties of the genome in the sense that they both describe the potential or propensity to vary (Wagner & Altenberg, 1996). Therefore, both canalization and developmental stability need to be analysed by comparison with a reference state (usually the wild type, Waddington, 1957). Consequently, insight into the relationship between canalization and developmental stability can be achieved by analysing the congruence between changes in the different components of

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phenotypic variation influenced by each property from a reference to a 'disturbed' state (Waddington, 1957; Klingenberg & McIntyre, 1998; Debat *et al.*, 2000; Réale & Roff, 2003). The genetic basis of both properties is believed to be partly rooted in coadapted gene complexes (favourable epistasis; Dobzhansky, 1970; Graham, 1992; Clarke, 1993; Leamy, 2003) that are organized at the population level. When individuals from genetically divergent populations are crossed, coadapted gene complexes may be disrupted, and an increase in the phenotypic variation is expected in hybrid progenies via a decrease in canalization and/or developmental stability. Thus, analysing the changes provoked by hybridization in within- and among-individual phenotypic variance, may allow inference of possible relationships between canalization and developmental stability (Debat *et al.*, 2000).

However, evidence for a negative effect of hybridization (either intra- or inter-specific) on developmental stability is conflicting (Graham, 1992; Clarke, 1993; Alibert & Auffray, 2003). Several hypotheses can be suggested to explain these inconsistent results. First, the breakdown of coadapted gene complexes can remain hidden at the F1 due to overdominance, and only the study of the F2 and F3, after recombination, will reveal a decrease in developmental stability (e.g. Andersen *et al.*, 2002). Inconsistencies may also result from the difficulties we have in measuring developmental stability. Fluctuating asymmetry (FA, subtle nondirectional departures from perfect bilateral symmetry; Van Valen, 1962) has been widely used as a measure of the phenotypic effects of developmental noise and consequently used to assess developmental stability (Palmer & Strobeck, 1986). However, variation in FA confounds the variation in developmental noise and the individual differences in developmental stability. Therefore, using variation in FA to measure variation in developmental stability remains rather imprecise, as a large component of the variation in FA is due to developmental noise (Whitlock, 1996, 1998; Houle, 1997, 2000; Van Dongen, 1998; Fuller & Houle, 2003; Pélabon *et al.*, 2004). Although in plants, measurement of homologous traits on repeated organs such as leaves and flowers can be used to estimate the within-individual phenotypic variance (Paxman, 1956; Freeman *et al.*, 2003), FA remains the measure least affected by macro-environmental variation. Indeed, the within-individual variance estimated by measures of repeated homologous organs will reflect changes in both environmental canalization and developmental stability, because of environmental variation encountered by different meristems or by a single meristem at different times.

It is therefore possible to identify different components of phenotypic variation, nested into each other, that are differently affected by environmental canalization and developmental stability. FA results from the phenotypic effects of developmental noise, and can thus be used to measure developmental instability (Nijhout & Davidowitz, 2003). The within-individual variation, which can be estimated by measuring repeated traits on modular organs, comprises developmental instability, positional variation, and imperfect homology of the modules. The among-individual variation comprises additive and nonadditive genetic variation, macro-environmental variation, and the corresponding $G \times E$ interactions, as well as the previously mentioned sources of variation. Developmental stability will therefore primarily affect FA and the within-individual variation by decreasing the effect of developmental noise, while environmental canalization will reduce both the within- and among-individual variations, depending on the amplitude of environmental variation at these two levels. Therefore, simultaneous analyses of changes in FA and phenotypic variation at both the within- and among-individual levels should allow us to infer relationships between environmental canalization and developmental stability (Hoffmann & Woods, 2001; Réale & Roff, 2003).

In the present study, crosses within and among four populations of the neotropical vine *Dalechampia scandens* (Euphorbiaceae) were used to analyse the effect of inter-population hybridization on environmental canalization and developmental stability. We first tested whether developmental stability, measured by FA, and environmental canalization, measured by the within- and among-individual variation, were associated with the genetic distance between parental populations and the degree of outbreeding depression observed in hybrids. Secondly, to test whether developmental stability and environmental canalization were controlled by common genetic mechanisms, we analysed the congruence of FA and components of phenotypic variation across parental and hybrid lines.

Interpretation of studies investigating the effect of hybridization on developmental stability using inbred lines as parental populations is often obscured by the potential effect of heterozygosity on developmental stability (Lerner, 1954; Mitton & Grant, 1984; Vøllestad *et al.*, 1999). By using natural populations with substantial molecular genetic variation revealed by ISSR analysis (W.S. Armbruster, T.F. Hansen, C. Pélabon, M.L. Carlson, J. Archibald & A. Wolfe, unpublished data), we avoid such confounding effects.

Methods

Study organism and breeding conditions

The neotropical, bee-pollinated vine *Dalechampia scandens* has unisexual flowers aggregated into bisexual pseudanthial inflorescences or 'blossoms' (Webster & Webster, 1972; Webster & Armbruster, 1991). Each blossom typically contains 10 staminate flowers arranged in three groups of three flowers around a

terminal flower. Three pistillate flowers subtend the staminate flowers. Associated with the staminate flowers is a gland, composed of bractlets that secrete terpenoid resin (Armbruster, 1984). Two large, showy involucral bracts subtend the groups of pistillate and staminate flowers plus the gland. Bees that collect the resin as nest-building material pollinate the flowers. The amount of resin offered to the pollinators depends on the total size of the gland and determines the size of the bee that can afford to visit the blossom (Armbruster, 1984, 1993). Consequently, the gland size as well as the distances between the gland and the stigma (GSD) and the gland and the anther (GAD), appear to be locally adapted to the size of the available pollinators (Armbruster, 1985, 1990; Hansen *et al.*, 2000).

The parental populations used in this study were derived from seeds collected at two locations in Mexico and two in Venezuela, early in 1998. All these populations are genetically and morphologically distinct (Fig. 1, see Hansen *et al.*, 2000 for locations). The Mexican populations, Chetumal and Tulum (Mex-1 and Mex-2, respectively), have large resin-producing glands, while the Venezuelan populations, Tovar and Caracas (Ven-1 and Ven-2, respectively), have smaller glands. Seeds were collected and stored by maternal family. Several seeds from each family were germinated in March–May 1998 at the greenhouse at the Department of Biology, NTNU (Trondheim, Norway). These plants were used as parental populations for the various crosses. Leaf tissues from several individuals were collected from each population for ISSR analysis (W.S. Armbruster *et al.*, unpublished data).

Conditions in the greenhouse were maintained as constant as possible during the whole experiment with an average temperature of 28 °C during the day and 22 °C during the night, 60–80% humidity and 13 : 11 h light : dark regime. Parental and F1 plants were fertilized weekly after they had their secondary set of leaves. Crosses were performed from September 1999 until February 2000 (see Hansen *et al.*, 2003a for description of manual crossing).

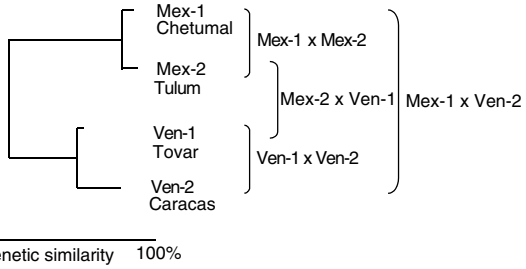

Fig. 1 Dendrogram of the genetic similarities between parental populations of *Dalechampia scandens* (on the left), and crossing design (on the right) for the inter-population hybridization. The genetic similarities are derived from ISSR analysis (percentage of shared bands; W.S. Armbruster *et al.*, unpublished data) of the different populations. For example, the Venezuelan and Mexican groups share on average 37% of their bands, while the two Mexican populations share an additional 49% of their genetic information. Note that none of the branch tips for each population reached 100% genetic similarity due to the genetic variation presents at the population level.

Crossing design

Four different types of hybrid seeds were produced. Two hybrid lines were produced by crossing distant populations (Mex-1 × Ven-2 and Mex-2 × Ven-1), further referred to as between-region hybrids. Within-region hybrids were produced by crossing nearby populations (Mex-1 × Mex-2 and Ven-1 × Ven-2; Fig. 1). Other possible crosses between distant populations were not conducted due to time and space limitations. Furthermore, within-population cross-pollinations were performed as control groups for each parental line. For each type of hybrid cross, we intended to obtain an equal number of progenies using each population as dam and sire. Subsequently, we tested for an effect of the direction of the cross on trait mean, variance and FA. Hybrid and parental lines were grown together in the greenhouse and experienced the same environmental conditions.

Seeds were germinated between 17 and 28 April 2000. Details of the seedling maintenance are reported in C. Pélabon, M.L. Carlson, T.F. Hansen & W.S. Armbruster (unpublished data). Plants were haphazardly moved in the greenhouse roughly every 2 weeks to reduce positional effects. Blossoms were measured from September 2000 until May 2001.

Germination success was fairly poor for the two parental lines from Ven-1 and Ven-2. To achieve a sufficiently large sample size, we measured individuals from the parental populations still present in the greenhouse at the same period. Differences between the parental populations and the F1 in trait mean, variance and FA were tested. The only significant differences were found in the Ven-2 population for the involucral bract measurements; the parental population had larger bracts than the offspring (mean ± SE: Parents: UBL = 16.22 ± 0.42 mm, LBL = 17.72 ± 0.43 mm, UBW = 17.45 ± 0.47 mm, LBW = 18.40 ± 0.48 mm; offspring: UBL = 14.05 ± 0.37 mm, LBL = 15.41 ± 0.45 mm, UBW = 15.40 ± 0.45 mm, LBW = 16.02 ± 0.50 mm, see Fig. 2 for traits definition and abbreviations). Therefore, hybrid mean and variance, for these traits, were compared with mean and variance of the offspring population only.

Measurements and measurement error

We measured 11 traits (Fig. 2a,b) with differing levels of modularity (see Hansen *et al.*, 2003b), on two haphazardly collected blossoms per plants. The between-region hybrids

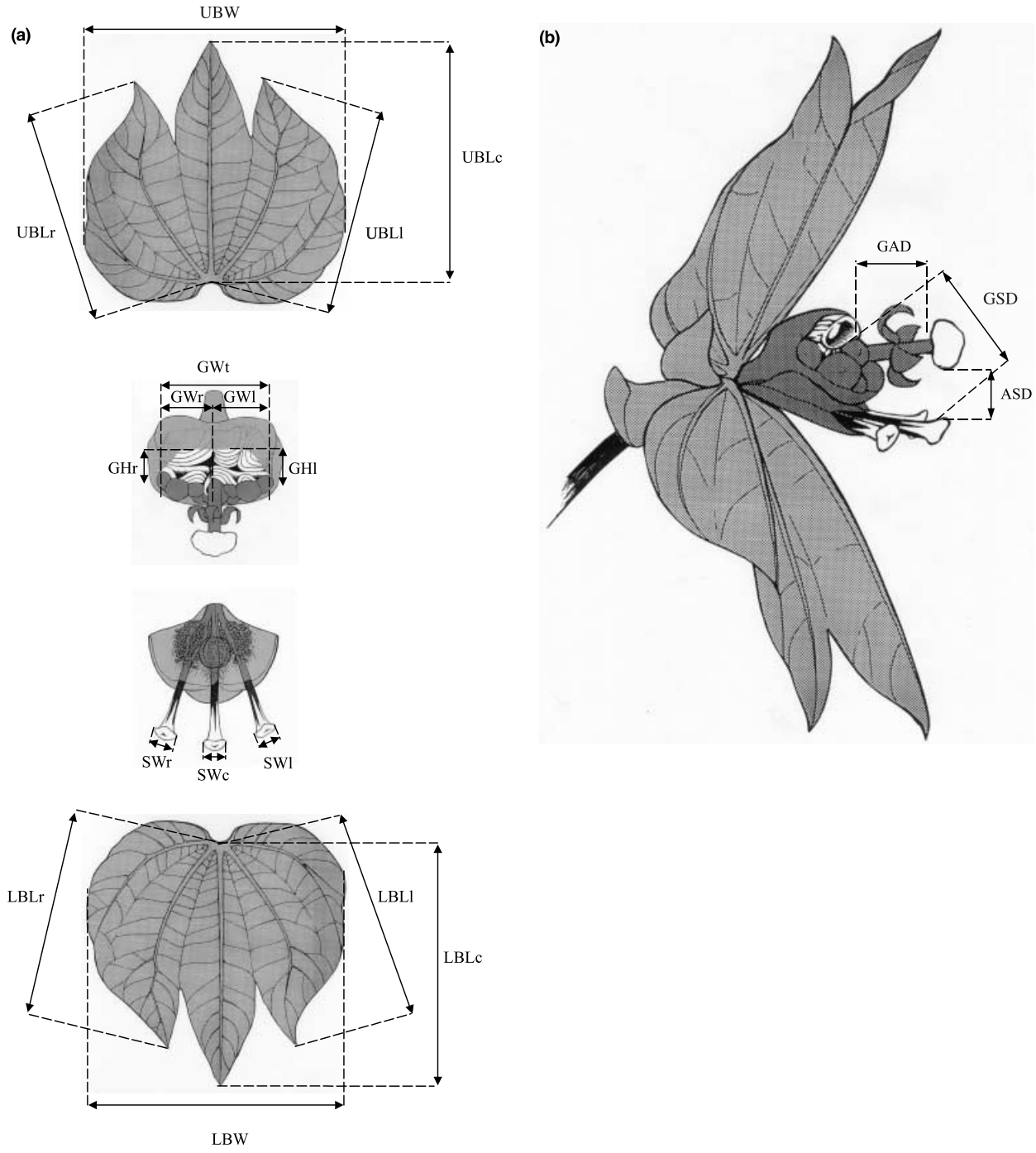

Fig. 2 Measurements on the *D. scandens* blossom. (a) Exploded view of the blossom illustrating the measurements on bilateral traits. UBW, upper bract width; UBL, upper bract length; LBW, lower bract width; LBL, lower bract length; GW, gland width; GH, gland height; GA = GW × GH, gland area; SW, stigma width. FA has been calculated for all traits for which a left and right components were measured (lower case letter). (b) Side view of the blossom illustrating the measurements of functional traits. GAD, gland-anther distance; GSD, gland-stigma distance; ASD, anther-stigma distance.

(Mex-1 × Ven-2) displayed substantially lower blossom production, preventing us from measuring the second blossom on some plants ($n = 10$). All measurements were performed by a single observer (CP) using an optical binocular magnifier (Optivisor®; Donegan Optical Co., Kansas City, MO, USA, 5× and 10× magnification).

We estimated FA of seven bilateral traits (Fig. 2) as follows (Clarke, 1998): $FA = 100\times[|\ln(L) - \ln(R)|]$, signed-FA referring to the signed difference $\ln(L) - \ln(R)$. The log-transformation of the data allows direct comparison of the FA level across traits and removes potential allometric effects on FA. To assess the magnitude of measurement errors on FA estimations, we performed repeated measurements. We then conducted two-way mixed-model ANOVA with the side as fixed factor and individual as random factor. For all traits, the interaction effect between side and individual was highly significant (all $P < 0.001$), thus showing that measurement errors were small enough compared with FA to carry out further analyses (Palmer, 1994). There was no correlation in FA among blossoms collected from the same plant (not shown). Therefore, all blossoms were used in the estimation of the FA level of each population.

Statistics

Under additive gene action, trait mean and variance of the hybrid progeny (F1) should be intermediate between those of the parental lines (Lynch & Walsh, 1998). Heterosis and outbreeding depression affecting developmental processes should result from nonadditive gene action such as underdominance and breakdown of favourable epistasis. Therefore, evidence of heterosis, outbreeding depression and changes in environmental canalization were analysed by comparing trait mean and variance of the hybrid progenies with the average mean and variance of the parental lines. We calculated the 95% CI of the difference between the hybrid mean or variance and the expected mean or variance (average of the parental lines) using bootstrapping. Because the variances were not independent of the mean values, we calculated variances on log-transformed data to be able to compare them when hybridization affected mean values.

The different types of progeny may differ in their level of FA on a trait-by-trait basis. However, a multivariate analysis that takes into account the different traits simultaneously should be a more powerful way to detect differences among cross-types in developmental stability (Zhivotovsky, 1992; Leung *et al.*, 2000). FA data were therefore subjected to both univariate and multivariate analyses. The multivariate analyses of FA comprised both a simple comparison of ranks and a discriminant analysis (see Juste *et al.*, 2001). Discriminant analysis is sensitive to underlying assumptions (Seber, 1984), in particular regarding homogeneity of the variance–covariance structure among lines. We first estimated the parameter of the Box–Cox transformation (Box & Cox, 1964) to get approximately normal distributions of the different variables. As likelihood-based intervals of the transformation parameter were centred on the value 1/3 for all variables, we used the cubic root of FA in the discriminant analyses. We furthermore compared the results obtained assuming identical variance–covariance matrices among crosses to those obtained assuming cross-specific matrices. Differences among populations in FA were assessed using Mahalanobis distances and the associated Hotelling's T^2 statistics for difference in mean values between each cross. All statistical analyses were performed in S-plus (Venables & Ripley, 1999).

For each trait, we calculated the differences in the level of FA, within- and among-individual variance, between hybrid and parental lines. Patterns of congruence among these differences across traits were tested to estimate the relationship between variational properties. Additionally, we also analysed the relationship between variational properties by calculating the line-mean correlation between FA and the two other sources of phenotypic variation for each trait (Réale & Roff, 2003).

A large number of statistical tests were preformed in this study, due in part to the large number of traits analysed. Because large numbers of statistical tests increase the probability of type I error (rejecting the null hypothesis when it is inappropriate to do so), Rice (1989) and others have suggested adjustment of α values. However, focusing on the α value of statistical tests may mask biological meaningful results (Yoccoz, 1991; Moran, 2003). In our study, the similarity of the trends (statistically significant or not) observed across traits is more important than the significance of each statistical test. Therefore, we used here *P*-values as relative measure of the evidence for an effect, not as error rates, as recommended by Cox (1977) or Berger (2003); see Shaffer (1995) for a discussion of the difficulties associated with multiple testing. Consequently, we did not correct the probability value for multiple tests in this study.

Results

Description of the outbreeding depression of between-region hybrids

Between-region hybrids, Mex-1 × Ven-2, displayed severe developmental disruption of both vegetative and floral traits. Normal internode elongation was suppressed resulting in a monopodial subshrub superficially similar to the normal growth form of species such as *D. spathulata* and *D. magnoliifolia*. The less extreme form consisted in the suppression of internode elongation at some growing points, with fairly normal internode elongation at other growing points, resulting in a stunted twining vine. The plants displaying the most extreme form of developmental disturbance did not produce blossoms.

Between-region hybrids appeared to be both male and female sterile. We performed several types of crosses including F1 × F1, backcross with the hybrid as sire or dam, and self-pollinations. None of these crosses resulted in the formation of seeds, and no seeds have been observed on F1 plants allowed to self-pollinate in the greenhouse.

The other between-region hybrids (Mex-2 × Ven-1) were even more severely affected. All but one hybrid offspring died within 2 months of germination. The seedlings measured at 1 month displayed an extremely low vigour (C. Pélabon *et al.*, unpublished data), and the only surviving individual displayed an extreme form of the syndrome described above.

Hybridization and trait mean

Non-additive gene action was apparent, as hybrid phenotypes were not always intermediate to those of the parental lines (Table 1). Hybrid progenies from the within-region cross between Mex-1 and Mex-2 showed trait mean that were greater than expected under additive model (significantly so in six of 11 traits) and closer to the mean value of the Mex-1 population (Table 1). Six traits from the hybrids between the Venezuelan populations, Ven-1 and Ven-2, were significantly smaller than expected (Table 1). None of the within-region hybrids displayed a maternal effect on the trait mean, but the likelihood of observing such an effect was low because nearby populations have rather similar blossoms.

A significant cross-direction effect on the trait mean was observed in the between-region hybrids Mex-1 × Ven-2 for the bract dimensions. Bracts of the individuals with a Ven-2 mother were significantly smaller than for those with a Mex-1 mother (Table 1). This suggests a maternal effect on bract dimensions, as the Ven-2 population has smaller blossoms than the Mex-1 population. For the remaining traits, hybrid mean values tended to be lower than the intermediate values between parental lines, the differences being significant in five of seven traits (Table 1). Note that none of the trait means of the hybrid progenies (all hybrid types included) was significantly larger or smaller than the corresponding trait mean in parental lines.

Hybridization and phenotypic variance

The total phenotypic variance in the within-region hybrids between the most genetically similar populations, Mex-1 and Mex-2, was sometimes higher (six traits) and sometimes lower (five traits) than the average variance of the parental lines, with a significant difference for one trait only (GW; Table 1, Fig. 3a). Hybrids from the other within-region cross, Ven-1 × Ven-2, consistently displayed phenotypic variances lower than the average variance of the parental lines, significantly so in five traits (Table 1, Fig. 3b). Progeny of the between-region cross, Mex-1 × Ven-2, displayed phenotypic variances greater than the average variance of the parental lines, significantly so for nine of 11 traits, and often also greater than the maximum variance observed in the parental lines (Table 1, Fig. 3c). Because of the effect of the cross-direction on the mean trait size (see above) and hence on the total phenotypic variance, we re-analysed the data using only the Ven-2 (sire) × Mex-1 (dam) cross ($n = 22$). In only one case (UBL) the new estimated variance was not significantly greater than the average parental line variance.

Hybridization and within-individual variance

The proportion of phenotypic variance expressed at the different levels of variation (within blossom – measured by FA, within individuals, and among individuals) was estimated using variance component analysis (Cox & Solomon, 2002). Within-individual variance represented the main source of phenotypic variance with an average of 55% (range: 14–91%) of the total variance explained by the difference among blossoms within plant. On average, the remaining variance was shared equally between the among-individual and the within-blossom (FA) levels, but there were strong differences among traits and populations. For example, GSD and GW in Mex-1 population had, respectively, 69 and 75% of their total variance explained at the within-blossom level (FA), while the variance in LBL expressed at this level never exceeded 5% of the total phenotypic variance in any crosses.

Changes in the within-individual variance between parental and hybrid lines followed the same pattern as changes in the total phenotypic variance. Within-individual variance decreased in Ven-1 × Ven-2 hybrids, and increased in the between-region hybrids (Mex-1 × Ven-2, Fig. 4b,c). No clear pattern was observed for Mex-1 × Mex-2 hybrids (Fig. 4a).

Hybridization and fluctuating asymmetry

Unlike phenotypic variance, FA was not affected by hybridization in a consistent way. Descriptive statistics of signed and unsigned-FA for the different lines are reported in Appendices 1 and 2, respectively. There was no evidence for directional asymmetry (systematic difference between the left and the right side), except in two cases (GSD in Mex-1 and GW in Mex-1 × Ven-2, Appendix 1). Furthermore, no distribution showed extreme skew, but some showed strong kurtosis as expected when the FA distribution results from the mixture of individuals experiencing different level of developmental noise (Rowe *et al.*, 1997). Thus, all bilateral traits conformed to a pattern of true FA.

Mean trait FAs were strongly correlated between pairs of lines ($0.71 < \rho < 0.96$). Considering each trait separately, lines displayed significant difference in their FA level (Appendix 2). Furthermore, when all traits were used to test for possible differences in FA among cross-types, large and significant (at 0.01 level) Mahalanobis distances were observed between several lines (Table 2). Therefore, the pattern of FA differed significantly among cross-types. However, there was no consistency across traits in the level of FA, and hybrid lines were not

Table 1 Trait mean and variance measured for the parental and hybrid lines. Trait mean values are in units of millimetre, and variances are calculated on ln-transformed data, and multiplied by 100 for the ease of reading. Mean and variance of the hybrid and the intermediate mean and variance of the parental lines were compared using bootstrapping. Deviance from the additive prediction is reported with the 95% CI. Data in bold correspond to the deviance where zero is not included in the 95% CI. For the Ven-2 × Mex-1 hybrids, we observed a significant effect of the cross-direction on the bract dimensions (see text). For these traits, mean values are reported separately for each cross-direction, with the statistics associated with the comparison.

Trait	Mex-1	Mex-2	Mex-1 × Mex-2	Ven-2	Ven-1	Ven-1 × Ven-2	Mex-1 (♀) × Ven-2	Ven-2 (♀) × Mex-1
N								
Individuals	30	36	42	30 (17/13)*	31 (11/20)*	23	22	7
Blossoms	59	72	57	60	62	46	39	9
UBL								
Mean ± SE	19.59 ± 0.28	17.00 ± 0.19	18.85 ± 0.22	15.35 ± 0.32	18.10 ± 0.30	15.66 ± 0.26	22.08 ± 0.88	16.94 ± 0.44
Deviance			0.53			**−1.08**	$F_{1,46} = 25.8$	$P < 0.001$
95% CI			−0.02; 1.07			**−1.73**; **−0.43**		
Variance	1.08	0.79	1.12	2.15	1.45	1.17	3.50	
Deviance			0.21			−0.61	**1.82**	
95% CI			−0.17; 0.61			−1.19; 0.14	**0.68**; **3.69**	
UBW								
Mean ± SE	21.84 ± 0.29	20.36 ± 0.23	21.84 ± 0.27	16.62 ± 0.36	19.73 ± 0.41	17.31 ± 0.30	21.85 ± 1.03	17.79 ± 0.55
Deviance			**0.73**			**−0.88**	$F_{1,46} = 10.6$	$P = 0.002$
95% CI			**0.10**; **1.34**			**−1.68**; **−0.16**		
Variance	0.95	0.81	1.18	2.36	2.18	1.28	4.16	
Deviance			0.32			**−1.00**	**2.43**	
95% CI			−0.04; 0.76			**−1.72**; **−0.11**	**1.00**; **4.57**	
LBL								
Mean ± SE	21.26 ± 0.33	18.46 ± 0.24	20.45 ± 0.25	16.79 ± 0.35	19.77 ± 0.38	16.93 ± 0.31	23.10 ± 0.89	18.15 ± 0.57
Deviance			0.60			**−1.34**	$F_{1,46} = 15.3$	$P < 0.001$
95% CI			−0.09; 1.17			**−2.19**; **−0.53**		
Variance	1.29	1.07	1.12	2.20	1.95	1.50	4.70	
Deviance			−0.04			−0.55	**2.95**	
95% CI			−0.51; 0.40			−1.25; 0.28	**1.11**; **5.64**	
LBW								
Mean ± SE	22.46 ± 0.33	21.08 ± 0.27	22.56 ± 0.31	17.44 ± 0.383	20.13 ± 0.46	18.16 ± 0.32	23.69 ± 1.32	18.76 ± 0.70
Deviance			**0.77**			−0.63	$F_{1,46} = 9.6$	$P = 0.003$
95% CI			**0.074**; **1.51**			−1.41; 0.18		
Variance	1.14	1.11	1.43	2.48	2.86	1.40	6.54	
Deviance			0.33			**−1.27**	**4.64**	
95% CI			−0.10; 0.92			**−1.99**; **−0.38**	**2.34**; **8.00**	
GAD								
Mean ± SE	4.67 ± 0.09	4.65 ± 0.06	4.71 ± 0.06	3.61 ± 0.07	3.46 ± 0.06	3.53 ± 0.07	4.12 ± 0.11	
Deviance			0.06			0.00	−0.02	
95% CI			−0.08; 0.21			−0.13; 0.14	(−0.26; 0.21)	
Variance	1.75	0.92	0.89	1.35	0.94	1.00	2.40	
Deviance			−0,47			−0.15	0.85	
95% CI			−1.70; 0.04			−0.59; 0.55	(−0.34; 2.47)	
GSD								
Mean ± SE	4.85 ± 0.08	4.61 ± 0.06	4.81 ± 0.07	4.59 ± 0.09	5.56 ± 0.14	5.04 ± 0.09	5.02 ± 0.14	
Deviance			0.07			−0.03	**0.30**	
95% CI			−0.09; 0.23			−0.29; 0.19	**0.03**; **0.56**	
Variance	1.03	0.94	1.17	1.35	2.61	0.95	2.91	
Deviance			0.20			**−1.01**	**1.70**	
95% CI			−0.20; 0.66			**−1.64**; **−0.47**	**0.80**; **3.07**	
ASD								
Mean ± SE	3.98 ± 0.17	3.17 ± 0.11	3.79 ± 0.11	0.52 ± 0.10	0.91 ± 0.15	0.41 ± 0.08	1.36 ± 0.15	
Deviance			0.22			**−0.29**	**−0.89**	
95% CI			−0.05; 0.51			**−0.54**; **−0.07**	**−1.16**; **−0.52**	
Variance	5.82	4.93	4.26	16.74	25.04	10.89	21.52	
Deviance			−1.17			**−9.94**	**10.06**	
95% CI			−3.80; 0.64			**−17.04**; **−3.76**	**(2.98**; **18.98)**	

Table 1 Continued

Trait	Mex-1	Mex-2	Mex-1 × Mex-2	Ven-2	Ven-1	Ven-1 × Ven-2	Mex-1 (♀) × Ven-2	Ven-2 (♀) × Mex-1
GW								
Mean ± SE	7.86 ± 0.08	6.81 ± 0.07	7.56 ± 0.08	5.72 ± 0.09	6.50 ± 0.10	6.11 ± 0.08	6.19 ± 0.15	
Deviance			**0.23**			−0.01	**−0.60**	
95% CI			**0.04**; **0,45**			−0.21; 0.22	**−0.96**; **−0.32**	
Variance	0.45	0.63	0.85	1.11	0.94	0.66	2.15	
Deviance			**0.33**			−0.35	**1.35**	
95% CI			**0.13**; **0.58**			−0.75; 0.01	**0.83**; **2.01**	
GH								
Mean ± SE	3.58 ± 0.06	3.01 ± 0.05	3.50 ± 0.05	2.39 ± 0.05	2.61 ± 0.04	2.38 ± 0.04	2.59 ± 0.07	
Deviance			**0.21**			**−0.12**	**−0.39**	
95% CI			**0.10**; **0.33**			**−0.23**; **−0.01**	**−0.55**; **−0.23**	
Variance	1.19	1.33	1.00	1.14	0.98	0.73	2.16	
Deviance			−0.22			−0.33	**0.96**	
95% CI			−0.63; 0.25			−0.79; 0.03	**0.31**; **1.93**	
SW								
Mean ± SE	1.50 ± 0.03	1.44 ± 0.02	1.55 ± 0.02	1.05 ± 0.02	0.97 ± 0.02	0.95 ± 0.02	1.12 ± 0.02	
Deviance			**0.08**			**−0.06**	**−0.16**	
95% CI			**0.03**; **0.12**			**−0.11**; **−0.02**	**−0.21**; **−0.11**	
Variance	0.56	0.72	0.51	0.33	0.79	0.38	0.67	
Deviance			−0.12			−0.17	0.22	
95% CI			−0.34; 0.07			−0.38; 0.04	−0.06; 0.62	
GA								
Mean ± SE	28.30 ± 0.70	20.67 ± 0.56	26.72 ± 0.65	13.83 ± 0.45	17.12 ± 0.46	14.64 ± 0.38	16.50 ± 0.80	
Deviance			**2.24**			−0.82	**−4.95**	
95% CI			**0.04**; **3.95**			−1.82; 0.31	**−6.29**; **−2.93**	
Variance	3.62	4.80	4.43	5.34	11.93	3.17	11.93	
Deviance			0.35			**−1.90**	**7.24**	
95% CI			−1.24; 1.97			**−4.14**; **−0.24**	**4.49**; **12.15**	

*Numbers between parentheses correspond to the number of individual germinated for the experiment (left part) and the number of individuals implemented from the parental populations present in the greenhouse at the same time (right part).

consistently more or less asymmetric than parental lines (Fig. 5).

Relationship among variational properties

Differences in within- and among-individual variances between parental and hybrid lines tended to be positively correlated (Fig. 6a). However, we found no congruence between the differences in FA and the differences in the two other sources of phenotypic variation (Fig. 6b,c). Furthermore, except for GAD, all traits showed a strong correlation across lines for the within- and among-phenotypic variance (GAD: $r = -0.04$; all other traits $r > 0.74$). Again, no consistent correlation across lines was found between FA and the two other variance components (average $r = 0.25$; range: −0.57–0.75).

Discussion

Inter-population hybrids and environmental canalization

Phenotypic variation appears to be affected by inter-population hybridization in our study system. Mean-standardized within- and among-individual variances were lower in hybrids of intermediate genetic distance than in parental lines (Ven-1 × Ven-2). Phenotypic variances remained practically unchanged in hybrids between closely related populations (Mex-1 × Mex-2), but strongly increased in hybrids between genetically distant populations (Mex-1 × Ven-2). Also, most individuals from this last category showed severe outbreeding depression expressed by complete sterility and disruption of the internode elongation. This U-shaped relationship between the phenotypic variance and the genetic distance has been observed in several other cases (Levin, 1970 and reference therein, but see Edmands, 1999). It has been interpreted as the results of the interplay between the conflicting effects of heterosis and the breakdown of coadapted gene complexes (Dobzhansky, 1970; Levin, 1970).

Edmands (1999) observed a decrease in phenotypic variance in inter-population hybrids in the intertidal copepod *Tigriopus californicus* across a wide range of populations. She suggested that this decrease could result either from the masking of deleterious recessive alleles, a general reduction in genetic variance in hybrids when the differences between parental populations were fixed,

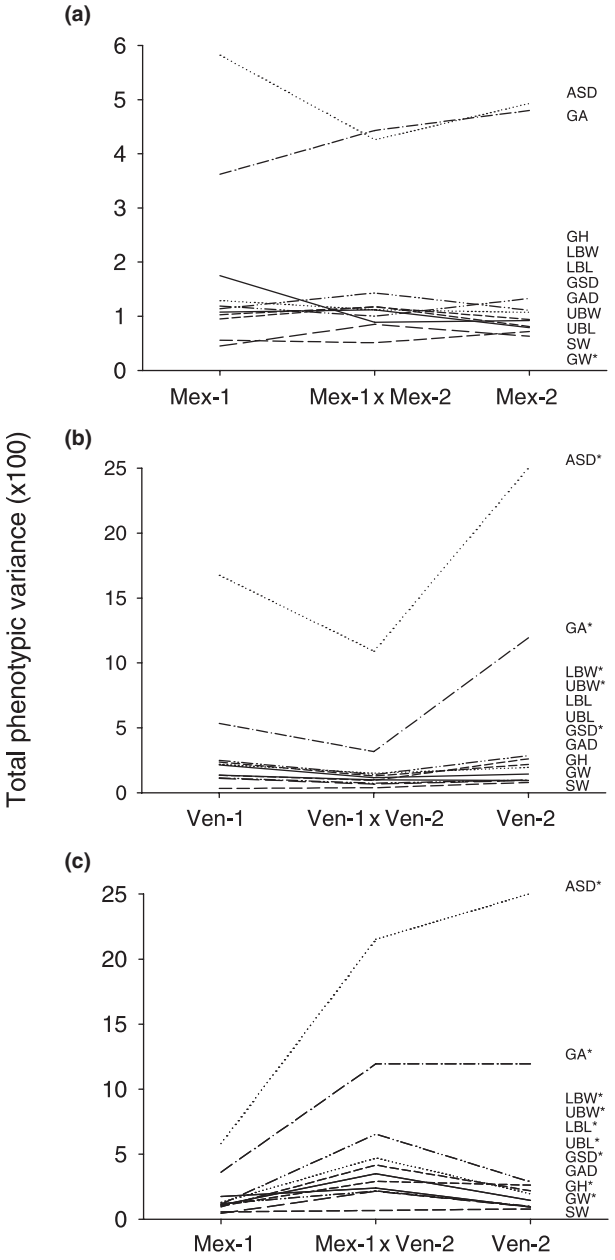

Fig. 3 Total phenotypic variance (100× variance on log scale) in parental and hybrid lines. (a) Mex-1 × Mex-2, (b) Ven-1 × Ven-2, (c) Mex-1 × Ven-2. Traits are listed in order of the magnitude of the trait's variance in the population at the right of the graph. Asterisks (*) indicate the traits for which the variance in the hybrid line is significantly different from the mean variance of the parental lines (zero not included in the 95% CI of the deviance).

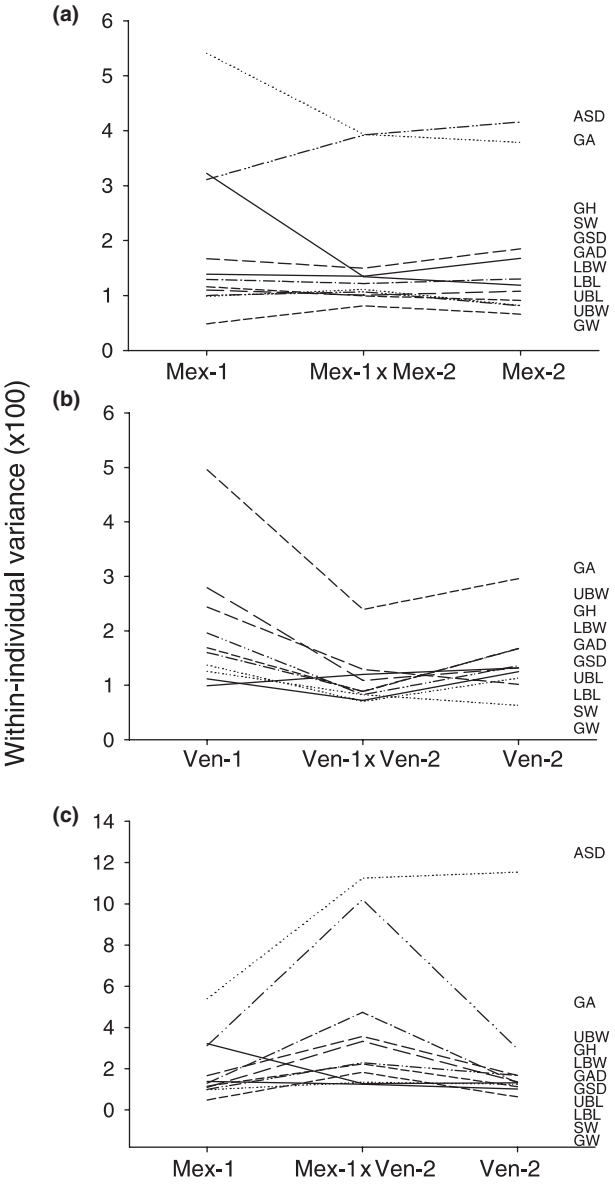

Fig. 4 Within-individual variance (100× variance on log scale) in parental and hybrid lines. (a) Mex-1 vs. Mex-1 × Mex-2: 4 increase, 7 decrease, Binomial test: $P = 0.55$; Mex-2 vs. Mex-1 × Mex-2: 6 increase, 5 decrease, Binomial test: $P = 1$. (b) Ven-1 vs. Ven-1 × Ven-2: 1 increase, 10 decrease, Binomial test: $P = 0.01$; Ven-2 vs. Ven-1 × Ven-2: 2 increase, 9 decrease, Binomial test: $P = 0.07$ (one trait, ASD, is not represented due to its extreme value, see Table 1). (c) Mex-1 vs. Mex-1 × Ven-2: 10 increase, 1 decrease, Binomial test: $P = 0.01$; Ven-2 vs. Mex-1 × Ven-2: 1 decrease, 9 increase, 1 unchanged, Binomial test: $P = 0.02$. Traits listed as in Fig. 3.

or an increase in developmental stability. Our study showed that the decrease in phenotypic variance in hybrids among populations of intermediate genetic distance resulted from decreases in both within- and among-individual variances. This result conflicts with the hypothesis of a reduction in genetic variance, because reduced genetic variance should not affect the within-individual variance. Furthermore, the absence of changes in FA with hybridization does not support the hypothesis of an increase in developmental stability.

Table 2 Mahalanobis distances between F1 lines (values associated with a T^2 statistic significant at the 0.01 level indicated in bold) for FA variables. Note that there was some evidence for heterogeneity in the covariance structure among groups (heteroscedasticity; adj. M test = 209.4, d.f. = 168, $P = 0.02$). Assuming a heteroscedastic covariance structure, the same five distances among populations were significant at the 0.01 level, and two additional distances (Mex-2 vs. Mex-1 × Mex-2 and Ven-1 vs. Mex-1 × Mex-2) were significant at this level.

	Mex-2	Ven-1	Ven-2	Mex-1 × Mex-2	Ven-1 × Ven-2	Mex-1 × Ven-2
Mex-1	0.28	**0.76**	0.33	0.52	0.54	0.46
Mex-2		**0.93**	0.52	0.43	0.44	0.34
Ven-1			0.53	0.49	0.31	**1.06**
Ven-2				**0.76**	0.49	0.43
Mex-1 × Mex-2					0.28	**0.87**
Ven-1 × Ven-2						0.50

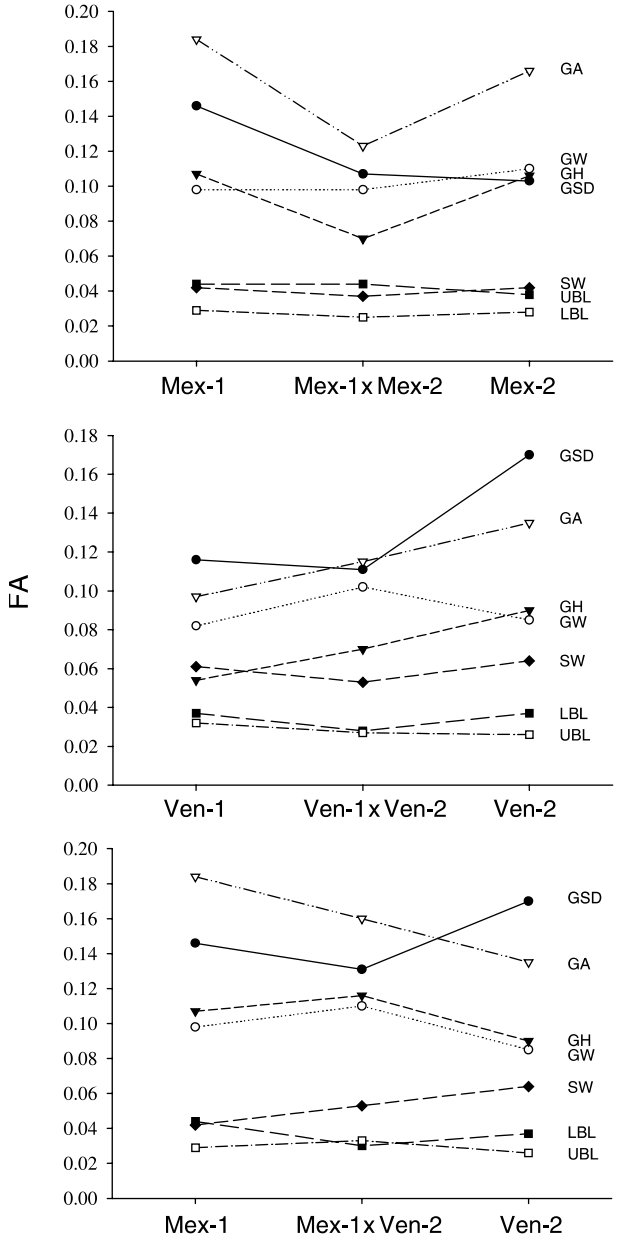

Fig. 5 FA in parental and hybrid lines. Traits are listed as in Fig. 3.

We suggest that the decrease in phenotypic variance observed at the F1 between weakly divergent populations results from a decrease in sensitivity to environmental variation, i.e. an increase in environmental canalization. Such an increase can result, either from the masking of deleterious recessive alleles or from an increase in favourable epistasis across chromosomes. Except in the case of fixed alleles, the masking of deleterious recessive alleles should mainly affect the among-individual variation while the increase of favourable epistasis should similarly affect both within- and among-individual variations. Furthermore, the decrease in canalization in F1 hybrids from genetically distant populations is usually attributed to a breakdown of coadapted gene complexes, i.e. the disruption of favourable epistasis (Lynch & Walsh, 1998). In this study, increases and decreases in within- and among-individual variances in hybrids already occurred at the F1, suggesting that modification in epistatic relationships across chromosomes (i.e. independently of recombination) was the main factor affecting the phenotypic variance via changes in environmental canalization.

Inter-population hybrids, FA and outbreeding depression

Despite significant differences among lines in the level of FA of several traits, no coherent changes in FA with inter-population hybridization were observed across traits, and hybrid progenies were not consistently more, or less, asymmetric than parental lines. These results are consistent with previous studies in which hybrids were either equally or less asymmetric than parental lines (Ferguson *et al.*, 1988; Alibert *et al.*, 1994; Freeman *et al.*, 1995; Gharrett *et al.*, 1999; Debat *et al.*, 2000; Andersen *et al.*, 2002; Rao *et al.*, 2002; see Alibert & Auffray, 2003 for review). However, in several of these studies, signs of heterosis were found in the F1 (e.g. Graham *et al.*, 1995; Gharrett *et al.*, 1999), suggesting that weak disturbances of coadapted gene complexes were masked by the beneficial effects of heterozygosity. Accordingly, Andersen *et al.* (2002) found a significant decrease in both fitness and developmental stability at the F2 and F3, after

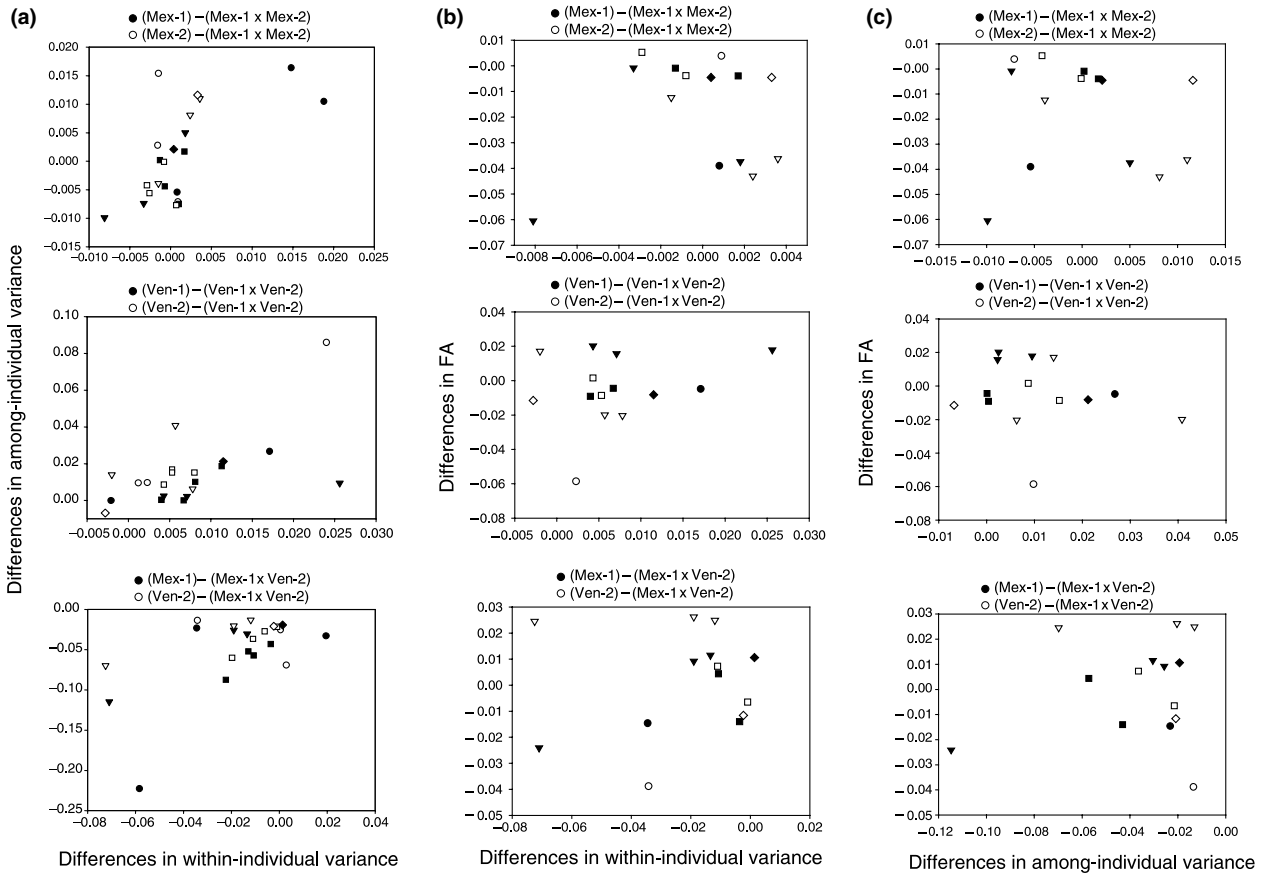

Fig. 6 Relationship between the differences in within- and among-individual variance and FA, between hybrid and parental lines. (a) Differences in within-individual variance vs. differences in among-individual variance. (b) Differences in within-individual variance vs. differences in FA. (c) Differences in among-individual variance vs. differences in FA. Each graph presents two sets of data corresponding to the differences in each trait between both parental lines and the associated hybrid line. Circle: functional traits GAD, GSD and ASD; square: bract dimension, UBW, UBL, LBW and LBL; triangle: gland dimensions, GH, GW and GA; diamond: SW.

having observed hybrid vigour at the F1 in intra-specific hybrids in *Drosophila mercatorum*. The severe outbreeding depression observed in hybrids between the most distant populations in our study system indicates, however, that the genetic disturbance was already strong in the F1. Therefore, the absence of a consistent increase in FA in these hybrids suggests, at best, a weak relationship between FA and the disturbance of coadapted gene complexes.

Nevertheless, we observed dramatic effects of hybridization between the most genetically distant populations on some developmental aspects such as internode elongation. Alados *et al.* (1998) used the deviation from the expected internode length as a measure of developmental instability in the mint (*Teucrium lusitanicum*), and showed a positive relationship between developmental instability and stressful conditions (see also Freeman *et al.*, 2003 for review). Internode variance may thus represent an alternative measure of developmental stability to FA. However, because the same meristem may encounter different environmental conditions during its growth, the variance in the internode elongation may also be affected by the sensitivity of the meristem to environmental variation. Therefore, internode elongation may often be more closely related to environmental canalization than to developmental stability.

Relationship between developmental stability and canalization

The hypothesis of a common mechanism acting simultaneously on FA and trait variance is implicitly acknowledged in models linking FA to developmental stability, where FA arises from random variance in the size on each side (Whitlock, 1996; Houle, 2000; Pélabon *et al.*, 2004). We found no correspondence in the differences between parental and hybrid lines in the levels of FA and in within- and among-individual phenotypic variance. This observation suggests that the mechanisms controlling developmental stability are not entirely the same as

those controlling environmental canalization (Waddington, 1957; Debat *et al.*, 2000; Hoffmann & Woods, 2001; Réale & Roff, 2003).

Nevertheless, FA can represent a large proportion of the phenotypic variance (up to 75% in GW), and traits showing large amount of FA tend also to be highly phenotypically variable (Clarke, 1998). One possible explanation suggested by Debat *et al.* (2000) and Rasmuson (2002) is that the functional importance of the trait leads to correlations between the different components of phenotypic variation, despite different regulatory mechanisms. This interpretation finds some support in the study reported by Rutherford (2000) on Hsp-90 in *Drosophila*, where the buffering of genetic and interaction (G × E) variance by Hsp-90 was not associated with changes in FA. Alternatively, Klingenberg & McIntyre (1998) suggested that despite similar genetic mechanisms controlling FA and phenotypic variance, different sensitivity of these mechanisms to environmental or genetic differences among individual and to random differences between body sides, may lead to the lack of congruence between FA and individual variance.

Because FA can represent a substantial source of phenotypic variation, it can play a major role in the ability of a trait to reach its adaptive peak by strongly affecting the accuracy of the trait's development. Although correlation between FA and phenotypic variance could occur due to congruent selective pressures (Rasmuson, 2002), the separation of the mechanisms controlling FA and environmental canalization suggests, at least theoretically, that FA and phenotypic variance may be affected by different sources of selection, and therefore may evolve separately.

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Appendix 1 Summary statistics for signed-FA [ln (*L*) − ln (*R*)]. Skewness and kurtosis of the distribution correspond to the Fisher's G1 and G2 respectively (*n*: number of blossom measured).

	Mex-1 ($n = 59$)		Mex-2 ($n = 72$)		Mex-1 × Mex-2 ($n = 57$)		Ven-2 ($n = 60$)		Ven-1 ($n = 62$)		Ven-1 × Ven-2 ($n = 46$)		Mex-1 × Ven-2 ($n = 48$)	
Trait	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.	Mean ±SE	Skew Kurt.
UBL	0.004	0.01	−0.001	−0.06	0.008	0.06	−0.004	0.07	−0.01	0.33	−0.004	−0.39	0.004	−0.33
	0.008	1.21	0.005	0.42	0.006	1.20	0.006	0.14	0.005	1.00	0.005	0.31	0.006	0.06
LBL	−0.004	0.39	0.003	−0.21	−0.0002	0.19	0.004	−0.02	0.002	1.263	−0.007	0.62	0.011	−1.95
	0.004	0.19	0.004	1.60	0.003	0.50	0.004	0.60	0.006	6.01	0.005	0.09	0.007	8.88
GSD	0.053	0.24	−0.018	−0.48	0.005	−0.20	0.019	−0.18	−0.008	−0.157	−0.005	−0.17	−0.02	−1.55
	0.023	0.22	0.015	0.49	0.015	0.26	0.031	1.06	0.02	1.202	0.021	1.18	0.027	3.17
GW	−0.013	−0.20	−0.019	0.47	−0.002	0.03	0.004	−0.21	−0.017	0.328	0.025	0.65	−0.046	−0.43
	0.01	1.28	0.016	1.90	0.013	−0.06	0.015	0.59	0.014	1.561	0.02	−0.00	0.02	1.10
GH	0.006	0.62	−0.006	0.32	−0.006	0.06	−0.005	0.50	0.004	−0.541	−0.018	−1.73	−0.026	1.01
	0.020	1.09	0.016	0.54	0.010	0.90	0.015	−0.07	0.009	3.286	0.015	5.19	0.021	4.11
SW	−0.001	−0.02	0.009	−0.92	0.002	0.42	0.010	−0.11	0.012	−0.514	0.001	−0.28	0.002	−0.04
	0.006	−0.16	0.006	4.18	0.005	0.16	0.011	0.21	0.01	0.37	0.009	−0.22	0.009	−0.44
GA	−0.007	−0.05	−0.026	0.24	−0.007	−0.06	−0.001	−0.10	−0.013	0.14	0.011	0.04	−0.073	−0.27
	0.032	1.17	0.023	0.19	0.018	0.67	0.022	−0.02	0.016	0.584	0.022	0.26	0.028	−0.34

Appendix 2 Summary statistics, mean (±SE) and CV, for FA calculated as: 100[| ln (*L*) − ln (*R*)|].

Trait	Mex-1	Mex-2	Mex-1 × Mex-2	Ven-2	Ven-1	Ven-1 × Ven-2	Mex-1 × Ven-2
UBL							
Mean ± SE	4.44 ± 0.54	3.82 ± 0.39	4.36 ± 0.39	3.69 ± 0.36	3.75 ± 0.35	2.83 ± 0.34	3.04 ± 0.30
CV	0.93	0.88	0.83	0.77	0.74	0.84	0.77
LBL							
Mean ± SE	2.85 ± 0.26	2.85 ± 0.32	2.47 ± 0.21	2.56 ± 0.29	3.16 ± 0.43	2.72 ± 0.30	3.29 ± 0.46
CV	0.70	0.95	0.79	0.88	1.07	0.78	1.08
GSD							
Mean ± SE	14.56 ± 1.53	10.27 ± 1.02	10.66 ± 0.92	16.99 ± 2.12	11.61 ± 1.26	11.13 ± 1.29	13.10 ± 1.73
CV	0.81	0.85	0.80	0.98	0.86	0.81	1.02
GW							
Mean ± SE	9.85 ± 1.22	11.01 ± 1.12	9.77 ± 0.75	8.52 ± 0.97	8.21 ± 0.96	10.23 ± 1.31	11.00 ± 1.23
CV	0.96	0.87	0.71	0.89	0.92	0.90	0.87
GH							
Mean ± SE	10.70 ± 1.40	10.58 ± 1.07	6.96 ± 0.71	9.00 ± 0.80	5.39 ± 0.65	6.97 ± 1.08	11.62 ± 1.29
CV	1.01	0.87	0.94	0.69	0.95	1.08	0.86
SW							
Mean ± SE	4.20 ± 0.40	4.20 ± 0.46	3.74 ± 0.33	6.42 ± 0.65	6.09 ± 0.95	5.27 ± 0.58	5.26 ± 0.48
CV	0.73	0.93	0.81	0.79	0.69	0.77	0.71
GA							
Mean ± SE	18.37 ± 2.20	16.61 ± 1.29	12.32 ± 1.16	13.51 ± 1.26	9.73 ± 1.00	11.52 ± 1.42	15.96 ± 1.66
CV	0.93	0.66	0.87	0.73	0.82	0.86	0.80