

EFFECTS OF CROSSING DISTANCE ON OFFSPRING FITNESS AND DEVELOPMENTAL STABILITY IN *DALECHAMPIA SCANDENS* (EUPHORBIACEAE)¹

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Crosses between genetically close and distant populations of *Dalechampia scandens* (Euphorbiaceae) were made to test whether the responses of various fitness components and measurements of developmental stability were affected by the outcrossing distance (level of outbreeding). Two fecundity traits, seed set and seed mass, decreased consistently with increasing level of outbreeding, and hybrids between the most divergent populations were sterile. Effects of the genetic distance between parental populations on viability traits, survival and vigor at 1 month of age, were highly idiosyncratic. Hybrids of one long-distance combination had no reduction in survival and vigor, while both traits were greatly reduced in the other long-distance combination. The expression of outbreeding depression on fecundity traits differed between reciprocal crosses in some hybrids but not others; thus, hybrid breakdown may have been due to cytoplasmic-by-nuclear gene interactions, reduced endosperm formation, or an interaction between progeny and maternal genotype. None of the measures of developmental stability had a consistent relationship with either genetic distance between parental populations or seedling vigor. These results suggest that fecundity and viability traits may be differentially affected by hybridization, probably due to differences in genetic architecture among populations. This study also confirms that developmental stability, as measured by the level of fluctuating asymmetry, is not a reliable index of genetic stress.

Key words: cytonuclear interactions; *Dalechampia scandens*; Euphorbiaceae; fluctuating asymmetry; genetic distance; interpopulation hybridization; outbreeding depression; outcrossing distance.

Interpopulation outcrossing often has positive effects on fitness-related traits. This heterosis effect is generally interpreted as the recovery from inbreeding depression caused by accumulation of deleterious alleles through genetic drift in isolated populations (Levin, 1978; Lynch, 1991; Mitchell-Olds, 1995; Keller and Waller, 2002; Hufford and Mazer, 2003). On the other hand, crosses between distant populations or species can also result in decreased fitness of the progeny, a phenomenon referred to as outbreeding depression (Price and Waser, 1979; Templeton, 1986; Lynch, 1991; Hufford and Mazer, 2003). Theoretical models of gene interaction therefore predict an optimal degree of outbreeding with offspring fitness decreasing on both ends due to inbreeding and outbreeding depression (Price and Waser, 1979; Lynch, 1991). Experimental studies, however, show very diverse relationships between fitness response and genetic or geographic crossing distance (Edmands, 2002). Some studies have shown large fitness advantages of F1 hybrids over a wide range of genetic or geographic distance (Edmands, 1999; Fenster and Galloway, 2000). In others, fitness declined with increasing genetic distance (Montalvo and Ellstrand, 2001), or fitness was highest when associated with intermediate crossing distance (Moll et al., 1965; Stacy, 2001; see Waser and Williams, 2001, for a review).

These observed inconsistencies may reflect the complexity of the mechanisms involved in the relationship between ge-

netic distance, genomic compatibility, and hybrid fitness. Outbreeding depression has been suggested to result from several mechanisms including dilution of locally adapted genomes with foreign genes (Templeton, 1986; Waser, 1993; Schierup and Christiansen, 1996; Montalvo and Ellstrand, 2001), underdominance (Schierup and Christiansen, 1996), and disruption of favorable epistatic interactions. This last is defined as the breakdown of coadapted gene complexes, also referred to as Dobzhansky-Muller incompatibilities (Dobzhansky, 1970; Lynch, 1991; see Coyne and Orr, 1998, and Burke and Arnold, 2001, for reviews). More dramatic chromosomal rearrangement such as chromosome inversion, translocation, or polyploidy may also affect the compatibility between parental lines (Lewis, 1973). The relationship between hybrid fitness and the genetic divergence between parental populations will thus depend on the mechanisms that generated this divergence.

If population divergence is mainly due to genetic drift and temporally fluctuating selection, subtle genetic changes should accumulate with time and geographic distance (Felsenstein, 1988; Hutchinson and Templeton, 1999). This pattern of divergence should result in a progressive reduction of hybrid fitness with an increasing genetic distance between parental populations, as epistatic interactions and underdominance become progressively affected by the genetic divergence between parents. Furthermore, the various trait components encompassing fitness should show, on average, identical responses to increasing genetic divergence. However, a trait-specific response is expected if the genetic architecture of the traits is differently affected during the divergence process, due for example to different types of selection. These include sexual vs. nonsexual traits (Coyne and Orr, 1997; Civetta and Singh,

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1998) and fecundity vs. viability traits (Turelli et al., 2001; Ramsey et al., 2003). In this case, reduction in trait optimality in hybrids with increasing genetic distance between parents will be inconsistent across traits, but should on average be related to the genetic distance. Major genetic modifications are likely to provoke complete and instantaneous prezygotic isolation without relation to geographic or genetic distances (Rieseberg, 2001). In this last case, hybrids from different crosses with similar genetic distance between parents could display very disparate fitness responses. Analyzing the relationship between the genetic distance between parents and the fitness response in different traits may therefore provide insights into the genetic mechanisms underlying population and species divergence and outbreeding depression.

Furthermore, if the genes involved in the outbreeding depression are nuclear and show Mendelian inheritance, the expression of the outbreeding depression in hybrids should be independent of the direction of the cross; that is, reciprocal crosses should not differ in their fitness (Tiffin et al., 2001). Consequently, asymmetries in outbreeding depression between reciprocal crosses should involve nuclear-by-cytoplasmic interactions (Galloway and Fenster, 1999; Tiffin et al., 2001) or interactions between the progeny genotype and maternal effect (Roach and Wulff, 1987).

Developmental stability is expected to decrease with outbreeding, as a consequence of the breakdown of coadapted gene complexes (Dobzhansky, 1970; Graham, 1992; Clarke, 1993; Alibert and Auffray, 2003), as well as with an increasing homozygosity (Lerner, 1954; Mitton and Grant, 1984; Palmer and Strobeck, 1986; Vøllestad et al., 1999). Fluctuating asymmetry (FA), subtle nondirectional departures from perfect bilateral symmetry (Van Valen, 1962), has been widely used to assess developmental stability (Palmer and Strobeck, 1986). However, the relationships of developmental stability (as measured by FA) with the breakdown of coadapted gene complexes and with heterozygosity are still unclear (Alibert and Auffray, 2003; Vøllestad et al., 1999). Furthermore, few studies have tested simultaneously the effect of hybridization on developmental stability and fitness components.

Here we present data on fitness-related traits and measures of developmental stability recorded in progenies of crosses within and among two Mexican and two Venezuelan populations of the vine *Dalechampia scandens* (Euphorbiaceae). Using hybrids between genetically close and distant parental populations, we tested whether viability and fecundity traits were similarly affected by increasing genetic distance between parental populations. We further compared the fitness performance of reciprocal crosses to test whether nuclear-by-cytoplasmic interactions or genetic-by-maternal effect interactions were involved in the mechanisms generating outbreeding depression. Finally, we tested whether fitness related traits and developmental stability display similar and/or correlated responses to an increasing genetic divergence between parental populations.

MATERIALS AND METHODS

Study organism and breeding conditions—*Dalechampia scandens* is a neotropical, bee-pollinated vine, with unisexual flowers aggregated into bisexual pseudanthial inflorescences or blossoms (Webster and Webster, 1972; Armbruster, 1985, 1990, 1991, 1993, 1996; Webster and Armbruster, 1991). Blossoms typically contain 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 that se-

cretes terpenoid resin (Armbruster, 1984). Two large, showy involucre bracts subtend the groups of pistillate and staminate flowers plus the gland. Bees that collect the resin for nest-building material pollinate the flowers. Each pistillate flower contains three ovules. After pollination, each blossom produces up to nine seeds. The capsules mature and dry out, and the seeds are dispersed by explosive dehiscence (Armbruster, 1982). Although highly divergent among *Dalechampia* species, the leaves are mostly three-lobed in *D. scandens* (Webster and Armbruster, 1991).

Individual plants used as parental stock were grown from seeds collected at two locations in Venezuela, Tovar and Caracas (Ven-1 and Ven-2, respectively) and two in Mexico, Chetumal and Tulum (Mex-1 and Mex-2, respectively), in early 1998 (see Hansen et al., 2000, for location of each population). Between 30 and 70 infructescences from separate individuals were collected from each population. Several seeds from each infructescence were germinated from March to May 1998 at the Biology Department greenhouse, Norwegian University of Science and Technology (Trondheim, Norway). 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 at night, 60–80% humidity, and 13 : 11 light : dark regime. All individuals were fertilized weekly after the seedlings produced their first true leaves.

Crossing design—We measured fitness components and developmental stability in progenies produced by crosses made within populations (parental lines), within regions (intermediate-genetic-distance hybrids) and between regions (long-genetic-distance hybrids). Because of time and space limitations, we conducted only two of the four possible between-region crosses (Mex-1 × Ven-2 and Mex-2 × Ven-1). Parental populations were used as both sire and dam for each type of cross. Within-population crosses did not include self-pollination. At least 30 successful crosses (15 in each direction for hybrid crosses), involving more than 20 different parents, were produced for each cross type. We maximized the number of individuals used as parents, and each individual plant was used a maximum of three times as dam or sire (median = 1).

The genetic similarity between parental populations was estimated by inter simple sequence repeats (ISSR) analysis. As part of a larger study on genetic diversity of *D. scandens* in Central and South America, we collected tissues from 12 plants grown from the field-collected seeds from each of the four parental populations. The analysis was based on five primers, and the average similarity has been calculated using a computer program written by Vera Ford (University of California, Davis). This program calculates the average similarity between groups where only band matches are used in the calculations (see Wolfe et al., 1998, for further details about the program). The genetic distances between parental populations reported in Fig. 1 were calculated as: $1 - \text{genetic similarity}$. Because ISSR analysis also allows estimation of the genetic similarities among individuals from the same population, within-population crosses have a non-zero genetic distance (Fig. 1). Also, the ISSR analysis revealed larger genetic variation (lower similarity between individuals) within the Tovar population (Ven-1) than existed between the two Mexican populations.

Between September 1999 and February 2000, mature, unopened blossoms were emasculated and the three stigmas were pollinated by applying pollen from a freshly opened male flower from the assigned sire. Care was taken to provide an excess of pollen on the stigmatic surface of each pistil. After pollination, the maternal blossom was labeled and bagged to prevent further uncontrolled pollination and to catch the mature seeds after 1 month.

Two sets of seeds were germinated between 17 and 28 April 2000. Each set comprised one seed per cross, haphazardly selected. Germination was induced by scarification and exposure to light and moisture in germination trays. After germination, seedlings from the first set, designated to be sacrificed for biomass measurements at 1 month, were planted 2 cm deep in perlite-filled 5-cm diameter pots. We choose to use a 100% perlite soil to be able to harvest and clean the roots at 1 month for biomass measurements. Seedlings from the second set, designated to grow to adulthood, were planted 2 cm deep in pots filled with a 1 : 1 perlite and sphagnum mixture. Upon planting, pots were placed in trays with regard to their set and germination date. Trays were

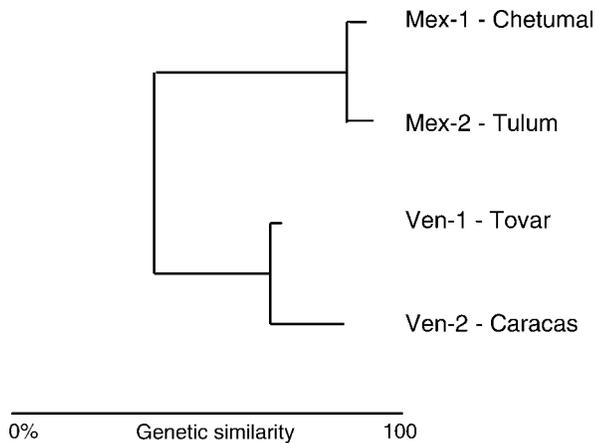


Fig. 1. Dendrogram of the genetic similarities between parental populations of *Dalechampia scandens*. Genetic similarities are derived from inter simple sequence repeats (ISSR) analysis of the different populations. For example, the Venezuelan and Mexican groups share on average 37% of their bands, while the two Mexican populations share an additional 49% of their bands. Note that none of the branch tips for each population reached 100% genetic similarity due to the genetic variation presents at the population level.

placed along a single bench, moved and rotated 90° every 2 days. At 10 and 24 days after germination, trays were filled with a solution of dilute fertilizer 2 cm deep to homogenize fertilization among pots. Some plants from the first set were kept alive and transplanted into a mixture 1 : 1 of sphagnum and perlite for further measurements at adult stage to compensate for the low germination success in the second set. Seedlings from the second set were transplanted into 15-cm diameter pots when they reached ca. 20 cm in height. These plants were grown to adulthood for measurements on leaves and blossoms (Pélabon et al., 2004a). During their growth and during the period of measurements, plants were regularly moved in the greenhouse to reduce positional effects.

Fitness measurements—Four fitness components, two related to fecundity and two related to viability, were measured for each cross type in both sets of plants. Fecundity traits were seed number per infructescence, and seed mass (measured before germination to the nearest 0.0001 g). We found no evidence for a trade-off between seed mass and seed number per infructescence (six correlations positive and six negative; average $R^2 = 0.047$; maximum $R^2 = 0.13$ for a positive correlation). Therefore, we did not include seed number per infructescence in the analysis of the seed mass. Viability traits were survival from germination to 1 month of age, and seedling vigor at 1 month (see later). Germination success was not analyzed because it may have been affected by variation in the scarification of the seeds.

When plants were 1 month old, we recorded the total number of leaves, the length of the two opposite primary leaves and the length of the three lobes of the largest leaf (Fig. 2) on all individuals. All measurements were performed using an optical binocular magnifier (Optivisor, Donegan Optical Co., Kansas City, Missouri, USA), at 5× magnification. Seedlings from the first set were subsequently removed from the perlite, and the roots were carefully cleaned. The plants were dried (48 h at 60°C) and weighed to the nearest 0.0001 g. The size of the largest leaf was strongly correlated with the seedling biomass (ANCOVA with cross-type as factor: leaf size $F_{1,96} = 492.12$, $P < 0.001$; cross type $F_{7,96} = 11.17$, $P < 0.001$; interaction $F_{7,96} = 0.55$, $P = 0.79$, R^2 increased from 0.28 to 0.85 when size of the largest leaf was included in the model). Therefore, we used the size of the largest leaf as an index of seedling vigor at one month of age in both sets of plants.

Fluctuating asymmetry measurements—The level of developmental stability for each cross-type was estimated at 1 month of age using two different FA measurements. The first one is the difference in length of the two opposite primary leaves (FA_{PL}) or first pair of true leaves. The second is the difference

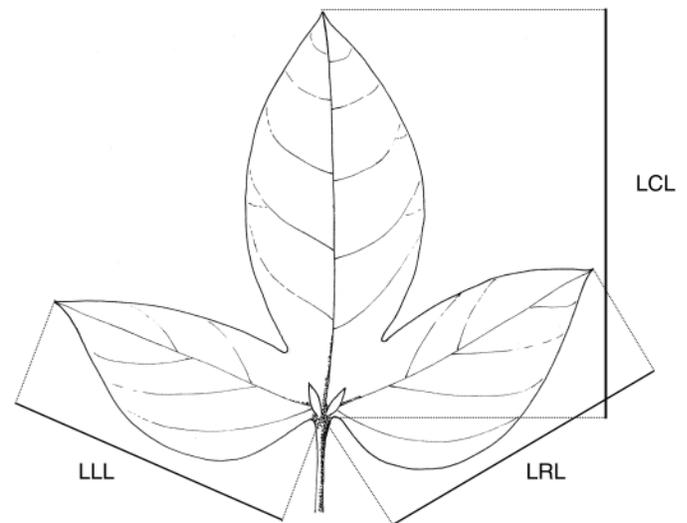


Fig. 2. Diagram showing the traits measured on a typical three-lobed leaf of *Dalechampia scandens* on adult plants and on the largest leaf of seedlings. LCL, length of the central lobe; LRL, length of right lobe; LLL, length of left lobe.

in length of the left and right lobe of the largest leaf (FA_{LL}) (Fig. 2). Both asymmetry measurements were positively correlated with the size of the leaves (FA_{PL} : $r = 0.14$, $N = 262$, $P = 0.02$; FA_{LL} : $r = 0.30$, $N = 268$, $P < 0.001$). Therefore FAs were calculated from log-transformed data ($FA_{PL} = 100(|\ln(\text{primary leaf 1}) - \ln(\text{primary leaf 2})|)$; $FA_{LL} = 100(|\ln(L) - \ln(R)|)$; Clarke, 1998). Signed FAs are the same measurements without taking the absolute value. Note that no signed FA was calculated for FA_{PL} , because a left and right primary leaf could not be specified. We also measured the leaf FA on adult plants from the second set (FA_{AL}). One leaf per plant was collected from a shoot in the outer part of the crown, and we recorded the same measurements as the ones recorded on the largest leaf at 1 month (Fig. 2) and calculated FA_{AL} .

Because of germination failure in the second set of plants, some cross types included only a few adult individuals. To improve balance and increase sample size, we measured FA_{AL} on individuals from the parental populations still present in the greenhouse in the same period or individuals from the same cross type, grown simultaneously in the greenhouse for parallel experiments. Before pooling the data, we tested whether FA differed between the initial and augmented data sets (see Results, Fluctuating asymmetry).

Measurement error and statistical analyses—To test whether measurement error affected our estimates of FA, we performed repeated measurements on live plants ($N = 16$) for all traits. Subsequent measurements were done within a few hours on the same day. We then ran a two-way mixed-model ANOVA with the side as fixed factor and individual as random factor (Palmer, 1994). For all traits, there was a significant interaction between side and individual ($P < 0.001$). Thus, measurement errors were sufficiently small (compared with asymmetry) to allow further analyses. We also corrected mean FA for bias caused by measurement error, as described in Pélabon et al. (2004b).

In the analysis reported later, “cross type” refers to the different crosses within and among populations produced in this study (eight different cross-types) and “cross direction” refers to the population of origin of the dam and sire in each cross. Because of maternal effects on seed production, all seed data were analyzed taking the cross direction into account. For survival, seedling vigor, and FA data, we first tested for an effect of the cross direction, and pooled data over reciprocal crosses if this effect was not statistically significant ($P > 0.1$). All statistical analyses were performed in S-plus (Venables and Ripley, 2002).

TABLE 1. Effect of the direction of the cross on the number of seeds per infructescence and the mass of the seeds produced by interpopulation crosses in *Dalechampia scandens* (one-way ANOVA). Crosses are presented in order of increasing genetic distance. The relative seed mass corresponds to the seed mass minus the average seed mass in the maternal population. Means (\pm SE) are given for each reciprocal cross, in the direction indicated in the first column (dam first) and then for the reciprocal cross (see also Fig. 3A, B).

Cross	df	F	P	Mean (\pm SE)
Number of seeds/infructescence				
Mex-1 \times Mex-2	1,37	0.87	0.36	7.09 (\pm 0.25)/6.73 (\pm 0.36)
Ven-1 \times Ven-2	1,58	9.19	0.004	6.24 (\pm 0.57)/7.47 (\pm 0.42)
Mex-1 \times Ven-2	1,73	4.16	0.045	5.52 (\pm 0.39)/6.45 (\pm 0.41)
Mex-2 \times Ven-1	1,50	2.98	0.09	5.96 (\pm 0.39)/4.70 (\pm 0.51)
Relative seed mass				
Mex-1 \times Mex-2	1,37	1.39	0.25	-0.0028 (\pm 8.5 \times 10 ⁻⁴)/-0.0043 (\pm 8.4 \times 10 ⁻⁴)
Ven-1 \times Ven-2	1,39	1.52	0.22	-0.0021 (\pm 4.3 \times 10 ⁻⁴)/-0.0009 (\pm 5.0 \times 10 ⁻⁴)
Mex-1 \times Ven-2	1,50	13.96	<0.001	-0.0125 (\pm 13.7 \times 10 ⁻⁴)/-0.0024 (\pm 8.2 \times 10 ⁻⁴)
Mex-2 \times Ven-1	1,41	0.25	0.62	-0.0030 (\pm 10.8 \times 10 ⁻⁴)/-0.0037 (\pm 4.1 \times 10 ⁻⁴)

RESULTS

Fecundity traits—In hybrid crosses, the direction of the cross significantly affected the number of seeds produced per infructescence, this effect being dependent on the cross type (Table 1, Fig. 3A). Furthermore, the number of seeds per infructescence varied greatly among the different cross types (ANOVA, $F_{11,388} = 12.76$, $P < 0.001$) and overall, significantly decreased with an increasing genetic distance between parental populations ($R^2 = 0.72$, Fig. 3A).

Seed mass of the hybrids was strongly correlated with the seed mass of the maternal population (regression, $F_{1,10} = 67.55$, $R^2 = 0.87$, $P < 0.001$). Therefore, we analyzed the relationship between seed mass and genetic distance, considering the difference in seed mass between the hybrid and the maternal population. In all cases, the seeds produced by a dam were the heaviest when sired by a plant from the same population, even when parental populations were genetically close, as in the case of Mex-1 and Mex-2 (Fig. 3B). The relative seed mass in hybrids (i.e., corrected for the maternal seed mass) was significantly affected by the direction of the cross in one of the interregion hybrids Mex-1 \times Ven-2 (Table 1). In general, seed mass tended to decrease with an increasing genetic distance between parental populations ($R^2 = 0.29$, Fig. 3B).

Manual crosses were made within each F1 hybrid lines. Within-region hybrids normally produced seeds (mean \pm SE number of seeds per infructescence: Mex-1 \times Mex-2 = 7.63 \pm 0.43, $N = 19$ crosses; Ven-1 \times Ven-2 = 7.05 \pm 0.46, $N = 18$ crosses). Between-region hybrids, however, produced fewer blossoms and were completely sterile even when backcrossed with the parental population as sire or dam (Pélabon et al., 2004a).

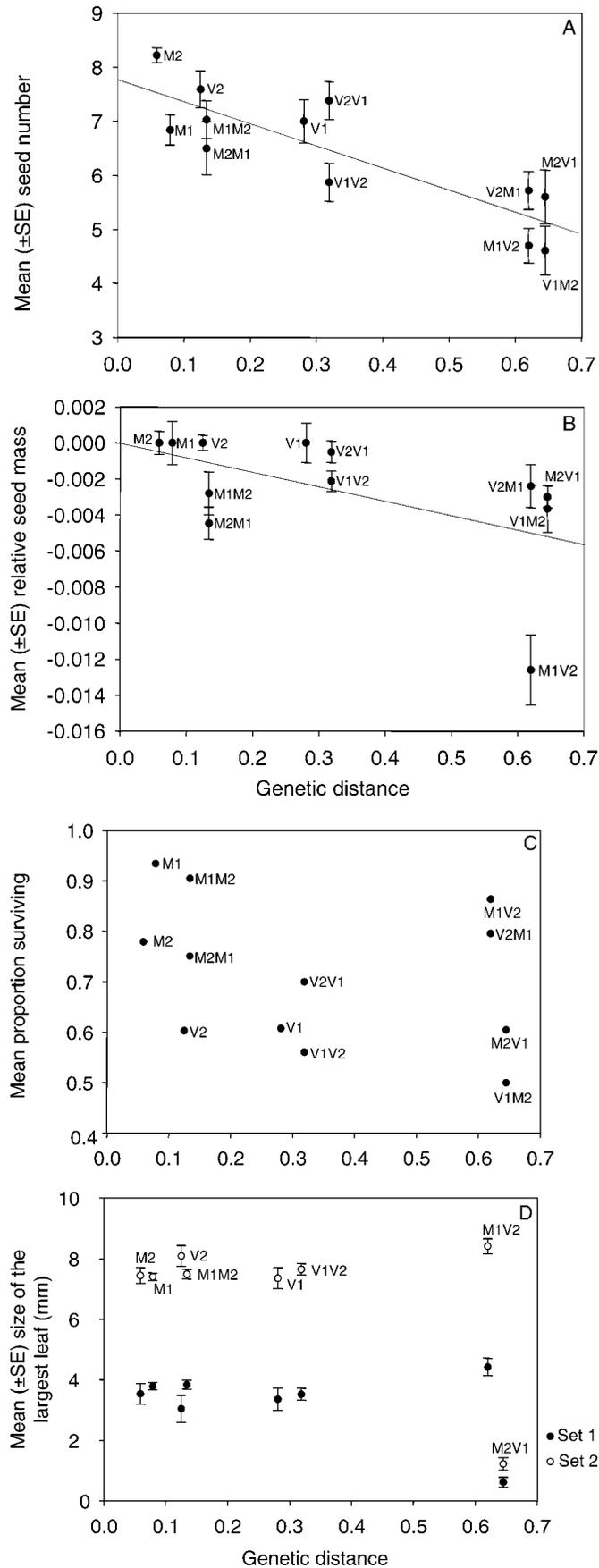
Viability traits—Survival from germination to 1 month of age differed among cross types ($\chi^2 = 38.76$, $df = 7$, $P < 0.001$), but did not differ between reciprocal crosses (not shown). Survival increased with seed mass in all cross types (Table 2), with no difference among sets of seedlings. The effect of genetic distance between parents on seedling survival was highly inconsistent, with one interregion hybrid showing reduced survival (Mex-2 \times Ven-1), while the other interregion hybrid did not show such an effect (Table 2; Fig. 3C). The low survival of the Mex-2 \times Ven-1 hybrids was later confirmed by the death of all but one individual within a few months.

The direction of the cross had no significant effect on seedling vigor estimated by the size of the largest leaf, whatever the cross type (not shown). However, seedling vigor differed among cross types but differently in the two sets of plants (Fig. 3D). Seedlings from the second set displayed greater vigor, with a maximum leaf size two times larger on average than the maximum leaf size of the seedlings from the first set (Fig 3D). This was presumably due to the presence of sphagnum in the soil of the second set of seedlings (see Methods, Crossing design). We found no consistent effect of genetic distance between parental lines on seedling vigor (Spearman's $R = -0.11$, $P = 0.66$, Fig. 3D). Interregion hybrids Ven-1 \times Mex-2 showed clear outbreeding depression with the size of the largest leaf significantly lower than the average value between parental populations (Fig. 3D). However, seedlings from the second between-region cross (Ven-2 \times Mex-1) did not display reduced vigor.

Fluctuating asymmetry—Descriptive statistics for FA measurements are reported in Table 3. All traits displayed the characteristics of true FA with symmetrical distributions around a mean of zero. Both FA measures recorded at 1 month of age differed significantly among cross types (ANOVA on square-root transformed data, FA_{LL} : $F_{7,255} = 3.94$, $P < 0.001$; FA_{PL} : $F_{7,248} = 2.75$, $P = 0.006$, Table 3) but without consistency, hybrids being neither more nor less asymmetric than parental populations. Hybrids from Ven-2 sire and Mex-1 dam had a higher level of FA_{PL} than hybrids from the reciprocal cross (Table 3), and this difference was an order of magnitude larger than the difference among cross types. Once this hybrid category was removed from the analysis, however, FA_{PL} did not differ among the remaining cross types (not shown).

Except for the hybrids Ven-2 \times Mex-1, FA in adult leaves (FA_{AL}) did not differ between individuals grown for this study and the set of complementary measurements done on other individuals from the same cross type grown simultaneously in the greenhouse (Table 3). For the hybrids Ven-2 \times Mex-1, the statistically significant difference in the level of FA_{AL} observed between the initial and complementary data did not affect the results (not shown). Therefore, we pooled the initial and complementary data sets for all cross types.

Leaf FA in adult plants did not differ significantly among cross-types (ANOVA on square-root transformed data, $F_{6,222} = 0.83$, $P = 0.54$, Table 3). None of the three FA measures was consistently affected by the genetic distance between pa-



rental populations (Fig. 4 A–C). Furthermore, the different FA measurements were not correlated with each other at the individual level (in all cases: $R < 0.08$, $P > 0.37$).

Finally, the hypothesis of a negative relationship between vigor and FA was not supported by our data. Although FA_{LL} tended to decrease with increasing vigor at 1 month, the effect size was extremely low ($R^2 = 0.02$), and this result was not supported by the other FA measurement (FA_{PL} , Fig. 5A and B).

DISCUSSION

Outbreeding depression and genetic distance—The various fitness-related traits responded differently to variation in the level of outbreeding. While the number of seeds per infructescence and seed mass tended to decline consistently with increasing genetic distance, survival and growth up to 1 month showed very idiosyncratic responses to the genetic distance between parental populations. Although both interregion hybrids had a fitness of zero, this outbreeding depression had very different causes, with one cross-type showing strong mortality before adulthood, while the other was completely sterile without any evidence of decreased survival or growth. Because this study was conducted in a common and noncompetitive greenhouse environment, it was not possible to estimate outbreeding depression due to local adaptation to environment (Dudash, 1990). Hence, the results obtained here are likely to be minimal estimates of outbreeding depression. However, these results, obtained in a common garden, clearly indicate a genetic basis for the observed outbreeding depression.

In a partially selfing species such as *D. scandens*, which is a colonizer of disturbed habitats, populations are likely to be founded by few individuals, leading to significant genetic drift. Selection and gene flow among adjacent populations may then restructure the drift-altered genome of substructured populations, by restoring coadapted gene complexes and increasing compatibility among nearby subpopulations. In such cases, populations may have somewhat different coadapted gene complexes, but more closely related populations sharing a more recent common ancestor would more often share similar gene complexes. This would result in a loose correlation between genetic distance and fitness, as observed here and elsewhere (Ritland and Ganders, 1987).

It is interesting that much of the outbreeding depression was expressed in fecundity traits such as seed production stage and fertility of adult plants, but not in viability traits (except for the hybrid Ven-1 \times Mex-2). Similarly, in a study on reproductive isolation between two *Mimulus* species (*M. lewisii* and *M. cardinalis*; Ramsey et al., 2003), hybrid crosses had lower

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Fig. 3. Relationship between various fitness components at the F1 and the genetic distance between parental populations of *Dalechampia scandens*. (A) Seed number per infructescence ($t = -5.16$; $df = 10$; $R^2 = 0.72$; $P < 0.001$). (B) Seed mass in g ($t = -2.00$; $df = 10$; $R^2 = 0.29$; $P = 0.07$; the data presented correspond to the seed mass in each cross type minus the seed mass in the maternal population). (C) Survival (see Table 2 for statistics). (D) Vigor at 1 month estimated by the size of the largest leaf; ANCOVA cross type \times set: $F_{7,254} = 4.76$, $P < 0.0001$, set: $F_{1,254} = 947.26$, $P < 0.0001$, cross type: $F_{7,254} = 68.13$, $P < 0.0001$; Abbreviation of the cross types: M1, Mex-1 (Chetumal); M2, Mex-2 (Tulum); V1, Ven-1 (Tovar) and V2, Ven-2 (Caracas). Cross direction is indicated as dam/sire.

TABLE 2. Analysis of the seedling survival from germination to one month of age in *Dalechampia scandens*. We performed an ANCOVA with the survival as response variable, seedling set as factor and seed mass and genetic distance as covariates (generalized linear model with a binomial error structure). Relative seed mass was defined as seed mass corrected for the effect of the cross type and cross direction. We selected the model using the AIC (Akaike information criteria, Burnham and Anderson, 2002), and the final model is indicated in bold. The coefficients (\pm SE) of the selected model are: intercept = 1.04 (\pm 0.13); mass = 71.98 (\pm 38.1), dispersion parameter = 1.15.

Model	AIC	Δ AIC	K	w_1
Mass \times set \times distance + mass \times set + mass \times distance + distance \times set + set + distance + mass	945.13	10.63	9	0.0021
Mass \times set + mass \times distance + distance \times set + set + distance + mass	944.68	10.18	8	0.005
Mass \times set + mass \times distance + set + distance + mass	942.79	8.29	7	0.016
Mass \times set + distance \times set + set + distance + mass	942.27	7.77	7	0.012
Set + distance + mass	940.95	6.45	5	0.042
Mass \times distance + set + distance + mass	940.32	5.82	6	0.031
Distance + mass	938.38	3.88	4	0.112
Mass	934.50	0	3	0.781

Notes: K = Number of estimable parameters; w_1 = Relative likelihood of the model (see Burnham and Anderson, 2002).

initial seed set and reduced pollen fertility and seed mass in adult plants, while plant growth and survival were not affected by hybridization. In *Impatiens capensis*, McCall et al. (1991) also found a decrease in the effect of the geographic distance between parents on fitness components expressed during later life-history stages. These authors suggested that distance between parents differentially affected fecundity and viability. Indeed, it can be hypothesized that directional selection is stronger for reproductive traits, especially in insect-pollinated plants, than for vegetative traits due to larger differences between adaptive optima. In this case, outbreeding depression is expected to increase more rapidly with the genetic distance between parents in reproductive traits than in vegetative traits.

Blossoms from different populations of *D. scandens* vary extensively in size, partly in response to the selective pressures imposed by various species of resin-collecting bees and by competition with other *Dalechampia* species for pollinators (Armbruster, 1985; Hansen et al., 2000, 2003a, b). Because *Dalechampia* communities and pollinator fauna are more variable on a geographic scale than is the abiotic environment of *D. scandens* (Armbruster, 1985), selective pressures on reproductive traits are expected to vary more strongly within and across regions than are selective pressures on viability traits. These expectations are consistent with the pattern of outbreeding depression observed in reproductive and vegetative traits in the present study.

Under the scenario discussed, increasing genetic divergence implies increasing variation in the fitness response as well as a decrease in the relationship between genetic and geographic distances (see case I in Hutchinson and Templeton, 1999). Consequently, the fitness of interpopulation crosses will be increasingly unpredictable with an increasing genetic divergence, and compatibility between highly divergent populations (or species) and viability of the F1 hybrids will be increasingly due to chance events. For example, in a compatibility study between five sympatric species of *Sidalcea* (Malvaceae), Gisler (2003) found that when pollen loads were high, some seed set was possible even between species of different ploidy levels. Overall, seed set was reduced, but the F1s that did germinate generally showed no obvious fitness losses at later life stages (Gisler, 2003). Cytological analysis showed that high pollen loads increased the number of pollen with chromosomal or genetic abnormalities that made them compatible with the maternal species.

Note that in our study, no heterosis effect was found in hybrids from parental populations with short to intermediate

genetic divergence, suggesting that the studied populations do not suffer from inbreeding depression (Keller and Waller, 2002). Furthermore, we never observed evidence of fitness reduction in selfed individuals (Hansen et al., 2003a; Pélabon et al., 2004b). This is not unexpected given the mixed mating system of *D. scandens* (Armbruster, 1988), with most of the deleterious recessive alleles likely having been purged (Carr and Dudash, 1996; but see Armbruster and Rogers, 2004).

Nuclear-by-cytoplasmic and $G \times M$ interaction—For the fecundity traits, the expression of outbreeding depression depended on the direction of the cross in interaction with the cross-type (Table 1, Fig. 3). Because reciprocal hybrids will have similar nuclear genetic information in the absence of heterogametic sex determinism, asymmetries in the outbreeding depression among reciprocal crosses can result from nuclear-by-cytoplasmic gene interactions (Galloway and Fenster, 1999; Campbell and Waser, 2001). Cytoplasmic–nuclear interactions occur when the expression of the cytoplasmic genetic information, inherited via female gametes for most plants, depends on the nuclear background (Wright, 1968; Galloway and Fenster, 1999). These results, together with other studies showing cytonuclear gene interactions in interpopulation hybrids (Galloway and Fenster, 1999; Fenster and Galloway, 2000; Willett and Burton, 2003; Galloway and Etterson, 2004) suggest that the genetic divergence between populations can involve other mechanisms than the fixation of incompatible nuclear alleles (Dobzhansky-Muller mechanism; Tiffin et al., 2001). Note that the effect of the maternal environment (e.g., maternal tissues surrounding the embryo and endosperm) on the expression of the progeny genotype can also provoke asymmetry between reciprocal crosses (Roach and Wulff, 1987). Additionally, asymmetry could arise from maternal effects in seed provisioning, as two maternal gene copies to one paternal gene copy are involved in endosperm formation. Further investigations are necessary to distinguish between these different mechanisms. Nevertheless, the absence of such an asymmetry between reciprocal hybrids in the expression of viability traits, as observed in this study, further suggests that population divergence differentially affects the genetic architecture of reproductive and viability traits including possibly the non-nuclear genetic information.

Fluctuating asymmetry, outbreeding depression, and genetic distance—Fluctuating asymmetry in hybrids did not increase consistently with the genetic distance among parental

TABLE 3. Descriptive statistics of FA measurements for *Dalechampia scandens*. The direction of the cross in the hybrids Ven-2 × Mex-1 had a significant effect on FA_{PL} (ANOVA, cross direction $F_{1,46} = 9.79, P = 0.003$; set: $F_{1,46} = 1.12, P = 0.29$, interaction $F_{1,46} = 0.15, P = 0.70$). Therefore we report the descriptive statistics for both cross directions separately in interregion hybrids. Note that the absence of adult plants from the cross Ven-1 × Mex-2 prevented us from estimating FA_{AL}. Mean FAs are corrected for measurement errors (Pélabon et al., 2004b). Measurement errors represented 1.6% of the observed FA_{PL} and 12% of the observed FA_{LL} and FA_{AL}. No evidence was found to support directional asymmetry (0 included in the 95% CI of the mean signed FA) or antisymmetry (CV < 0.75, Pélabon et al., 2004b).

Cross-type	Mex-2		Ven-1	Ven-2	Mex-1 × Mex-2		Ven-1 × Ven-2		Mex-2 × Ven-1		Ven-1 × Mex-2	
	59	24			13	16	64	30	16	35	4	10
Signed FA _{LL}	0.01	1.48	2.54	2.33	0.40	-0.38	0.6	0.17	-0.7	-11.6		
95% CI	-1.45; 1.48	-1.65; 4.62	-10.83; 5.75	-1.07; 5.74	-0.83; 1.63	-1.90; 1.13	-4.6; 5.8	-1.9; 2.2	-16.0; 14.6	-44.0; 21.0		
Mean FA _{LL}	3.86	4.48	7.81	4.43	3.57	2.70	5.02	3.26	12.6	12.7		
CV (FA _{LL})	0.79	1.08	1.18	0.88	0.62	0.85	1.41	1.288	1.056	1.24		
Mean FA _{PL}	6.21	6.06	10.38	9.74	11.70	7.42	20.01	5.75	12.3	9.84		
CV (FA _{PL})	0.78	0.87	1.30	0.78	1.85	1.15	1.20	0.99	1.045	0.95		
N	31 ^a	20 ^a	27 ^a	32 ^a	42	24	13	40 ^a	—	—		
Signed FA _{AL}	0.87	0.86	1.08	-0.55	1.66	0.65	3.60	1.10	—	—		
95% CI	-1.89; 3.63	-1.32; 3.04	-1.09; 3.25	-3.47; 2.36	-0.39; 3.72	-1.26; 2.55	-1.49; 8.70	-1.15; 3.76	—	—		
Mean FA _{AL}	4.84	3.43	3.89	5.06	4.38	3.15	6.03	5.03	—	—		
CV (FA _{AL})	0.93	0.66	0.75	0.98	0.93	0.77	0.86	1.06	—	—		

^a Samples completed with additional plants from the same population or cross type grown for other studies. No difference in FA_{AL} was observed for any cross type, except for one: Mex-1, $t = 0.66, P = 0.51$; Mex-2, $t = -0.16, P = 0.87$; Ven-1, $t = 1.54, P = 0.13$; Ven-2, $t = -0.36, P = 0.72$; Ven-2 × Mex-1, $t = 2.17, P = 0.03$, mean FA_{AL} (CV) sample 1: 7.74 (0.93); sample 2: 4.85 (1.01).

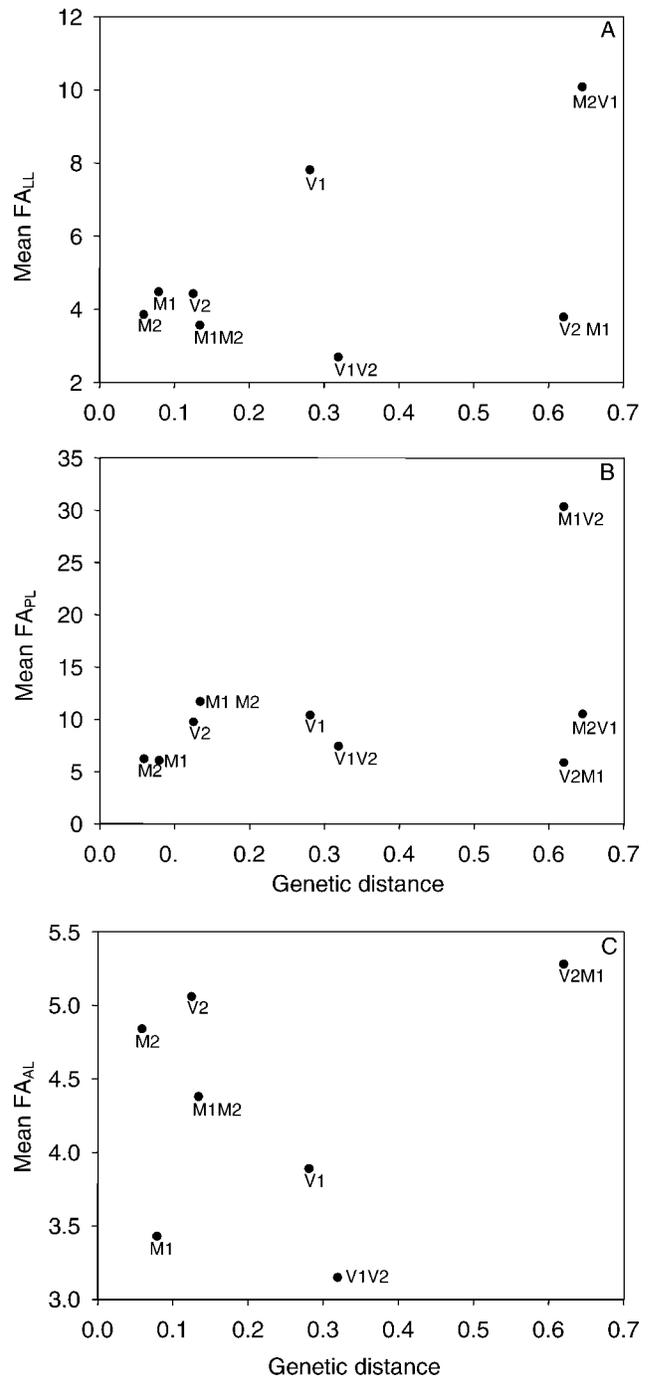


Fig. 4. Relationship between the genetic distance between parental populations of *Dalechampia scandens* and measures of developmental stability in the F1: (A) FA in largest leaf, (FA_{LL}), (B) FA in primary leaves (FA_{PL}), (C) FA in adult leaf (FA_{AL}). Data were pooled over reciprocal crosses when FA measurements were not affected by the direction of the cross (all cases except for FA_{PL} in Mex-1 × Ven-2).

lines. Many studies have failed to detect any clear relationship between FA and hybridization (Alibert et al., 1994; Freeman et al., 1995; Gharett et al., 1999; Rao et al., 2002). Several of these studies have also failed to detect any significant difference between hybrid and parental fitness (Freeman et al., 1995; Gharett et al., 1999). In these cases, it is possible that the

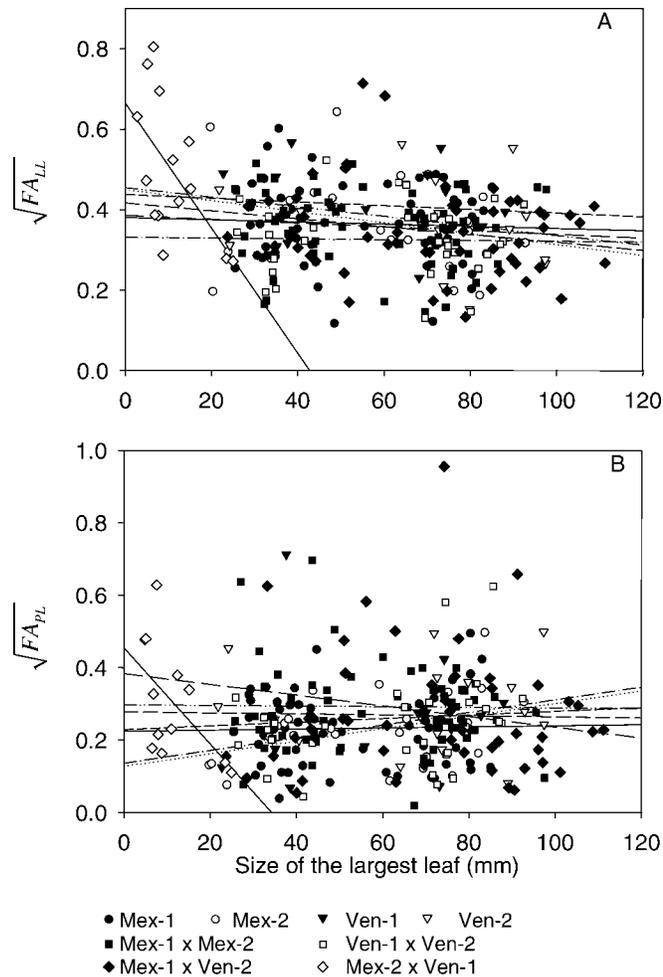


Fig. 5. Relationship between seedling vigor estimated by the size of the largest leaf (mm) and measurements of developmental stability (square-root transformed) in *Dalechampia scandens*. (A) FA of the largest leaf (FA_{LL}), (B) FA in primary leaves (FA_{PL}).

disruption of gene coadaptation caused by hybridization was insufficient in the F1 to provoke any substantial decrease in developmental stability (Andersen et al., 2002). The strong outbreeding depression observed on reproductive traits in between-region hybrids in our study indicates, however, that the genetic distance between parental populations was large enough to produce significant genetic stress. Nevertheless, only one of three FA measurements responded weakly to this genetic disruption. Thus, FA does not appear to be a reliable indicator of genetic stress in plants. These results also support the general belief that the control of FA is a trait-specific property (but see Polak et al., 2003) and elicit questions about the cause of differential sensitivities of different FA traits to hybridization.

Developmental stability of traits showing the highest genetic divergence across populations or species, such as reproductive traits, may be more sensitive to hybridization than traits that have diverged less (Civetta and Singh, 1998). Waldmann (1999) also suggested that developmental stability of vegetative traits is less sensitive to genetic disturbance than is developmental stability of floral traits because leaves have higher developmental plasticity. We found, however, no support for

these predictions. Using individuals from the second set, we studied FA on seven different blossom traits and found no consistent changes in FA with hybridization (Pélabon et al., 2004a), suggesting that developmental stability in reproductive traits is no more sensitive than it is in vegetative traits (Wilsey et al., 1998; Hochwender and Fritz, 1999; Perfectti and Camacho, 1999).

Our results indicate that there is at most a weak relationship between fitness components of the F1 and the genetic distance between parental populations. However, part of the observed variability seems to be attributable to the difference between fecundity and viability traits in their response to varying levels of outbreeding. This can be expected in animal-pollinated plants, where more rapid divergence of the genetic architecture in fecundity than in viability traits may result from the selective pressures related to prezygotic isolation or the use of different pollinators in geographically distant populations. This appears to be the case in our study system (Armbruster, 1985; Hansen et al., 2000). The present study also supports the conclusion that the response of FA measurements to genetic disturbance generated by hybridization is highly unpredictable (see also Lamb et al., 1990; Clarke, 1993; Rao et al., 2002), and FA measurements are not more sensitive to hybridization than fitness-related traits. Despite analyzing numerous FA traits and the extreme variation in fitness between different cross-types, we could find no relationship between these variables.

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