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LIFETIME FITNESS IN TWO GENERATIONS OF *IPOMOPSIS* HYBRIDS

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Various models purporting to explain natural hybrid zones make different assumptions about the fitness of hybrids. One class of models assumes that hybrids have intrinsically low fitness due to genetic incompatibilities, whereas other models allow hybrid fitness to vary across natural environments. We used the intrinsic rate of increase to assess lifetime fitness of hybrids between two species of montane plants *Ipomopsis aggregata* and *Ipomopsis tenuituba* planted as seed into multiple field environments. Because fitness is predicted to depend upon genetic composition of the hybrids, we included F1 hybrids, F2 hybrids, and backcrosses in our field tests. The F2 hybrids had female fitness as high, or higher, than expected under an additive model of fitness. These results run counter to any model of hybrid zone dynamics that relies solely on intrinsic nuclear genetic incompatibilities. Instead, we found that selection was environmentally dependent. In this hybrid zone, cytoplasmic effects and genotype-by-environment interactions appear more important in lowering hybrid fitness than do intrinsic genomic incompatibilities between nuclear genes.

KEY WORDS: **Fitness, hybridization, natural selection, reproductive isolation.**

Natural hybridization can have a variety of evolutionary consequences. These include a stable hybrid zone, fusion of genetically distinct taxa into a single species, reinforcement of species differences, and evolution of new hybrid species. In large part, which trajectory is followed depends on how fit hybrids are relative to their parental species. For example, low fitness of hybrids due to strong intrinsic genomic incompatibilities would prevent fusion into a single species and could instead select for reinforcement of species differences (Servedio and Noor 2003). Because of its central role in reproductive isolation and speciation, the fitness of hybrids has been debated at least since the evolutionary synthesis (Dobzhansky 1937). One class of models for the dynamics of hybrid zones assumes that hybrids have intrinsically low fitness (Barton and Hewitt 1985), whereas another class assumes that fitness is not necessarily low and may depend strongly upon the environment (Anderson 1948; Endler 1977; Arnold 1997). In the latter case, a greater variety of evolutionary dynamics are possible, including invasion of novel habitats by relatively fit hybrid individuals (Arnold 1997).

The ideal test of hybrid fitness and its dependence upon the ecological setting would include several elements. First, hybrid fitness would be measured in multiple environments (Rieseberg and Carney 1998; Hatfield and Schluter 1999), allowing one to determine if the expression of fitness varies greatly with environment or whether low fitness is intrinsic. Second, fitness would be estimated across most or all of the life history, as snapshots of fitness components over short periods can be misleading (Miglia et al. 2005). Third, the fitness of several classes of hybrids would be included, as fitness can depend on hybrid genotype. In particular, one important model for hybrid fitness and its role in speciation posits that second-generation hybrids (F2 hybrids) will often show reduced fitness. This "hybrid breakdown" can be attributed

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to genetic incompatibilities that derive from epistatic interactions between alleles (Dobzhansky 1937; Muller 1942). In this scenario, a mutant allele has become fixed at the A locus in one species, whereas another mutant allele has become fixed at the B locus in a second species. Some F2 hybrids will be homozygous for both of these mutant alleles. Because both allele substitutions evolved in separate allopatric populations, there would have been no selection for them to function well in combination, and F2 hybrids may have low fitness as a result. F1 hybrids, in contrast, may enjoy relatively high fitness (although this is not assured), because heterozygosity and thus any heterosis are greatest in that generation. Examination of such Dobzhansky–Muller incompatibilities has usually focused on epistatic interactions between nuclear genes, but interactions between nuclear genes and uniparentally inherited cytoplasmic genes such as chloroplast and mitochondrial genes may also contribute (Turelli and Moyle 2007; Ellison and Burton 2008). Differences between reciprocal crosses can provide evidence for cytonuclear interactions (Levin 2003).

Plants are particularly well-suited to tests of hybrid fitness, as it is possible to conduct experiments in which individuals of known genotype are planted into field environments and followed to obtain estimates of fitness. Several studies have now examined performance of hybrid plants in multiple field environments (Emms and Arnold 1997; Wang et al. 1997; Fritsche and Kaltz 2000; Miglia et al. 2005; Rhode and Cruzan 2005). A few field transplants have also included later generation hybrids (Rhode and Cruzan 2005; Fritz et al. 2006; Johansen-Morris and Latta 2006) allowing for tests of epistatic effects on fitness components, although reciprocal effects are rarely tested. And, a very few have measured lifetime fitness through an entire generation from seed to seed (Campbell and Waser 2007).

The goal of this article is to report on a study involving all three of these elements—multiple environments, lifetime fitness estimates, and examination of different hybrid classes. We used hybrids between *Ipomopsis aggregata* (scarlet gilia) and *I. tenuituba* (Polemoniaceae). These are sister species (Grant and Wilken 1986; Wolf et al. 1993), with little genetic divergence (Wu and Campbell 2005). They form natural hybrid zones in some, but not all, areas of contact (Aldridge 2005). Previous studies with these species have compared the relative fitness of F1 hybrids and the parental species, using the finite rate of increase as a measure of lifetime female fitness (Campbell and Waser 2007). In the current study, we perform an entirely new experiment that also uses this measure of fitness (Lande 1982; McGraw and Caswell 1996) and repeats the planting of first-generation reciprocal F1 hybrids and parental types, but differs in the addition of F2 hybrids and backcrosses. The primary objective is to measure the lifetime fitness of these different types of hybrids in multiple field environments. We ask: (1) Is there hybrid breakdown in lifetime female fitness in the second generation, as measured under field conditions? (2) Does the fitness of first- and second-generation hybrids differ between ecological settings? We measured fitness in two settings: in the center of a natural hybrid zone and in an environment at one end of the zone, where only one parental species is found.

Materials and Methods

STUDY SYSTEM

Our study site was a hybrid zone in Poverty Gulch, Gunnison County, Colorado. Plants of *I. aggregata* spp. *aggregata* grow in the valley bottom at elevations of 2900 m and below, plants of *I. tenuituba* spp. *tenuituba* grow on steep slopes above 3100 m, and natural hybrids grow on open talus slopes in between (Grant and Wilken 1988; Wu and Campbell 2005). At Poverty Gulch, plants of both species are perennial monocarps that usually germinate in the summer following seed production, spend 2–10+ years as a basal rosette of leaves, flower during a single season, set seed, and then die. Seed dormancy is rare; in one study 96% of those that germinated did so in the year following seed production (Campbell 1997). Plants rarely live longer than 10 years (e.g., 3% in Campbell and Waser 2001). Monocarpy and lack of seed dormancy allowed us to measure fitness over the entire life history.

Whereas the two species have similar life histories, they differ conspicuously in floral traits. Flowers of *I. aggregata* spp. *aggregata* have relatively short, wide, and red corolla tubes. Those of *I. tenuituba* are relatively long, narrow, and white to pale-colored, and secrete much less nectar than their congener (Meléndez-Ackerman 1997). At our study site, the main pollinators of both species are hummingbirds, with hawkmoths visiting only in rare years (Campbell 2004). Flowers are highly self-sterile (Waser and Price 1991). Despite growing at higher elevations, *I. tenuituba* and hybrid plants experience higher maximum temperature and lower relative humidity during the summer than plants at the valley bottom, due to rocky, scantily vegetated surfaces. *Ipomopsis tenuituba* plants and hybrids also have correspondingly higher photosynthetic water-use efficiency than do plants of *I. aggregata* from this region (Campbell et al. 2005; Wu and Campbell 2007).

CROSSING DESIGN

This study was initiated in 1995 when we performed crosses by hand to generate the F1 hybrids that would be required as parents for an experimental F2 generation. We collected 10 parental plants of each of the two species from opposite ends of the hybrid zone outside the range of natural hybridization (sites C and L in Campbell et al. 1997). Plants were potted and brought to the Rocky Mountain Biological Laboratory (RMBL), where flowers were pollinated by hand in a screenhouse. Each *I. aggregata* plant

was crossed to a different *I. tenuituba* plant to yield 10 independent full-sibling families. F1 progeny from these crosses were raised in pots at the RMBL, kept in screenhouses during summers, and sunk into the ground inside gopher-proof fencing during winters. Plants were watered as needed and fertilized twice a summer with 20–20-20 NPK fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH) (1 T/gal Peter's Professional).

In 1997 and 1998 many F1 individuals flowered, allowing us to make crosses to produce various F2 classes. It was not possible to generate sufficient progeny of all classes in a single year, because the number of F1 plants flowering was limited, as were the numbers of *I. tenuituba* and hybrid plants we could collect in nature to serve as parents for backcrosses, without depleting natural populations. In 1997 we generated seeds to plant into a site (site I in Campbell et al. 1997) in the center of the natural hybrid zone. We began with this site because it is in such sites that seeds of diverse genetic backgrounds, including F2 hybrids, would most likely be formed. In 1998, we generated a new batch of seeds to plant into the *I. aggregata* parental site (site L), to test whether results would be general across these two sites. We recognized that this design would confound site with year of planting. Nevertheless it tested whether relative fitness differed between two different ecological conditions (whether due to site or time differences). To determine the sensitivity of relative fitness to time differences, we compared results obtained here for our parental types and F1 hybrids to those seen in a previous study in which seeds were planted in 1994 simultaneously into parental and hybrid sites (Campbell and Waser 2007). If results for those first-generation hybrids are consistent between years, then it is likely that any differences seen are due primarily to site effects.

The 1997 experiment included seeds of the following nine types: (1) *I. aggregata* × *I. aggregata* (AA); (2) *I. tenuituba* × *I. tenuituba* (TT); (3) F1 hybrid from interspecific crosses with *I. aggregata* as the maternal parent (AT); (4) the reciprocal F1 hybrid (TA); (5) F2 hybrid from F1 × F1 crosses (HH); (6) backcross between *I. aggregata* and F1 hybrid (AH); (7) the reciprocal backcross (HA); (8) backcross between *I. tenuituba* and F1 hybrid (TH); and (9) the reciprocal backcross (HT). In all cases, the first letter indicates the maternal parent. We planned 270 crosses, as shown in Figure 1. All crosses except those for producing the F2 hybrids were performed as reciprocal factorials, and so each entry in the figure represents the crosses in both directions, i.e. with both plants serving as the maternal parent. We used a diallel design (without selfs) to produce the F2 hybrids, as we had a limited number of genetically independent F1 families from which to draw parents. Each of the five F1 plants used in a particular cross type came from a different full-sibling family (Fig. 1), and we used a total of 19 F1 individual plants, to maximize the genetic diversity of second-generation hybrids. Plants of the parental species were collected from the field sites described above. For

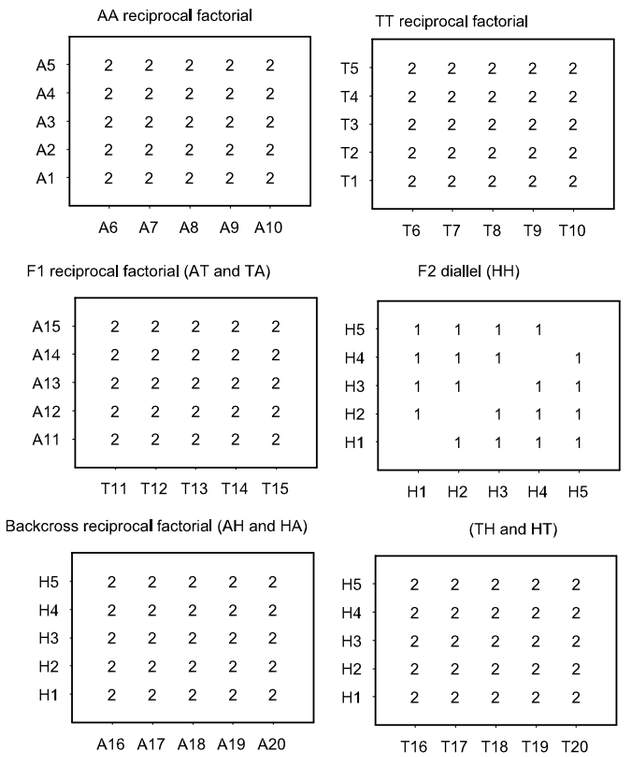

Figure 1. Crossing design used to produce the 270 crosses of nine types (AA, TT, AT, TA, HH, AH, HA, TH, and HT) planted as seeds into both sites. Additional reciprocal factorial crosses similar to those shown for AA were used to produce the 50 NN crosses. A1–20 and T1–20 indicate different individual parent plants of *I. aggregata* and *I. tenuituba* taken from natural populations in the summer when crosses were made. H1–5 indicate F1 hybrids from different full-sibling families, but plants with the same label are not always the same individual plant from that family. For the reciprocal factorial crosses two crosses were made (one in each direction), whereas for the diallel each entry represents a single cross, as indicated by the numbers.

each cross, we hand-pollinated up to five flowers. A few crosses (7 out of the 270) could not be made because appropriate flowers did not come into bloom simultaneously. Hand-pollinated flowers were individually labeled, and we collected fruits just before natural dehiscence of the seeds and counted their contents.

The 1998 experiment had a similar design, except that we added a tenth cross type (NN), consisting of seeds obtained from reciprocal factorial crosses between 10 natural hybrids collected that year from the natural hybrid site (site I). In total, we planned 320 crosses that year (270 as in 1997, plus 50 for NN), again with up to five hand-pollinations per cross. We were unable to complete nine of the 320 crosses because appropriate flowers did not bloom simultaneously.

EXPERIMENTAL PLANTINGS

In early September, after all seeds had been collected, we planted them into field sites, the natural hybrid site (site I) in 1997 and the *I. aggregata* site (site L) in 1998. Seeds were planted just under the soil surface at 10 cm intervals using a 1 m × 1 m planting frame gridded with fishing line. Corners of the planting frame were marked with large nails to allow relocation during subsequent censuses. Existing vegetation was not disturbed, except that we removed flowering individuals of *Ipomopsis* from within 1 m of planted areas to avoid input of seeds that might be confused with those we planted. This method allows reliable relocation of individual plants, as seedlings emerge in regular rows and columns, and flower traits of plants that survive are consistent with the genetic backgrounds of the seeds (Campbell and Waser 2007).

In both years we planted in a randomized block design with 10 blocks, each made up of three 1 m × 1 m squares. In general, each block contained one seed of each of the cross types, assigned at random, with the following modifications. For crosses that produced insufficient seeds, we planted instead a seed from its reciprocal cross. If a seed was unavailable from either reciprocal, a spot was left unplanted. For the 1998 planting, there were 311 crosses and only 300 spots in the grid for each block, but several crosses produced few seeds and so were not represented in all blocks. In total, we planted 1938 seeds in 1997 and 2704 in 1998.

FITNESS MEASUREMENTS

We censused the 1997 and 1998 plantings in late June or early July of each succeeding summer through 2007. For each individual seed planted, we recorded seedling emergence in the first summer following planting. In subsequent summers we recorded survival and stage (vegetative rosette or blooming). At the end of the season we collected fruits and the calyces from failed fruits every 2–3 days to estimate number of flowers made by each plant that bloomed, the seeds per flower, and total seed production (methods in Campbell and Waser 2007). Throughout the blooming season we minimized unwanted gene flow through pollen into surrounding populations by either emasculating all flowers while still in bud phase (Campbell and Waser 2001), or by collecting fruits from other *Ipomopsis* plants within a 5 m radius of our plots. That distance includes the vast majority of pollen-mediated gene flow (Campbell 1991).

Because the performance of offspring from the same plant may not be independent, we used maternal family as a unit of replication in analyses by combining all seed progeny of a particular mother for a given type of cross. In 1997 we had 55 maternal families: 10 AA, 10 TT, 5 AT, 5 TA, 5 F2, 5 AH, 5 HA, 5 TH, and 5 HT (Fig. 1). In 1998 we had 65 maternal families, including 10 of natural hybrid crosses (NN).

For each maternal family, we first determined mean seeds per pollination to assess the rates at which various hybrid types can be formed. Relative production of seeds from interspecific crosses versus conspecific crosses is one component of prezygotic reproductive isolation, as opposed to postzygotic isolation as reflected in hybrid fitness. To assess the fitness of hybrids, we therefore determined (1) survival of a seed to reproduction, (2) mean total seed production for blooming plants, (3) mean age at reproduction, and (4) the finite rate of increase (λ). The last measure (λ) is as an estimate of lifetime fitness that takes into account age-specific schedules of survival and fecundity (Lande 1982; McGraw and Caswell 1996). We were unable to measure male fitness because of the need to control genetic contamination through pollen dispersal, but previous studies have suggested that patterns of male and female reproductive success, in comparing parental species with hybrids, are similar (Meléndez-Ackerman and Campbell 1998). As an alternate measure of fitness that does not take into account age at reproduction, we also calculated net reproductive rate (R_0), the number of seeds produced per seed planted.

Seeds per pollination was first calculated for each full-sibling family and then averaged over the fathers mated with a particular female. Females with fewer than five hand-pollinations were dropped from the analysis. Survival, seed production per survivor, and age were calculated for the pooled group of seeds from a particular female (Campbell and Waser 2007). The few cases with fewer than five seeds planted were dropped from analysis. For the *I. aggregata* site only (for which we had more data on reproductive plants), we also analyzed two components of seed production per survivor: mean number of flowers produced, and mean seeds per flower. The finite rate of increase was calculated from age-specific survival and reproduction of seeds planted in the field by finding the dominant right eigenvalue for the Leslie projection matrix (Campbell and Waser 2007).

By the end of a 10-year period of growth in 2007, 193 plants had flowered and set seed. All but five of these (2.6%) died following seed set. For the five iteroparous plants, we used first age at blooming in our age analysis. An additional 16 plants (0.3% of the original 4642 seeds planted) remained alive at the end of 2007. As in previous studies, we assumed for analysis that each of these 16 plants would bloom during the next year and set a number of seeds equal to the average for its maternal family. Most plants that survive for more than 8 years do eventually flower, and relaxing this assumption even to the point of assuming zero survival makes little difference to the fitness estimates, because elasticity to events after year 10 is extremely low (Campbell 1997; Campbell and Waser 2007).

STATISTICAL ANALYSES

We first analyzed seeds per pollination, lifetime fitness of progeny (λ), and fitness components for maternal families belonging to the

nine cross types shown in Figure 1 (AA, TT, AT, TA, HH, AH, HA, TH, and HT), using analysis of variance (ANOVA) for each site separately. Each ANOVA was supplemented by orthogonal a priori contrasts specified in Procedure GLM of SAS to test specific hypotheses about hybrid fitness. Fitness components examined were survival to reproduction, seed production of reproductive individuals, and age at reproduction. The a priori contrasts included: comparisons between the two species, the average of the F1 versus the midparent of the species (a test for heterosis), the F2 versus the average of the F1 and the midparent (a test for hybrid breakdown reflecting interactions between nuclear genes), and the difference between AT and TA, the two reciprocal F1 hybrids. We included the last test because our previous studies had detected higher lifetime fitness, and higher survival, for AT than TA at the *I. aggregata* site, suggesting interactions between nuclear genes and either chloroplast or mitochondrial genes (Campbell and Waser 2007), both of which are inherited maternally in these species (Wolf et al. 1993). Because we had a priori predictions concerning the direction of the differences between the species and between reciprocal F1 hybrids at the *I. aggregata* site, we used one-tailed tests for the corresponding contrasts. For the *I. aggregata* site only, a separate ANOVA was used to compare the fitness of the natural hybrid progeny (NN) with the F2, to see whether natural hybrids have diverged from the array of recombinant types present in the F2.

We then assessed whether relative fitnesses were different across the two experiments conducted at different sites. For these tests, we combined the two datasets into a single ANOVA and set up several a priori contrasts, as follows. Based on previous studies (Campbell and Waser 2007), we asked whether fitness of AT—fitness of TA is more positive at the *I. aggregata* site, and whether fitness of AA—fitness of TT is more positive at the *I. aggregata* site, using one-tailed tests because our prior studies generated one-way predictions. In addition, we compared the level of heterosis between the two sites, and the level of hybrid breakdown, using two-tailed tests. Finally, we asked whether the difference between backcrosses to *I. aggregata* and to *I. tenuituba* differed between the two sites, which would indicate an interaction between the environment and the additive effects of genes, as shown by Rundle and Whitlock (2001).

We log-transformed data when they did not meet assumption of normally distributed residuals, which corrected the problem in most cases. At the hybrid site, residuals for the finite rate of increase, however, departed markedly from normality even after transformation, as they included a large number of zeroes. For this case, we performed randomization tests for each of the a priori contrasts. We wrote a macro in SAS (SAS, Cary, NC) to permute values at random and repeated this procedure 1000 times to generate a null distribution of the contrast for comparison with the actual contrast.

LINE CROSS ANALYSIS

For the experimental garden at the *I. aggregata* site we further analyzed lifetime fitness using line cross analysis (Lynch and Walsh 1998). We did not perform this analysis for the hybrid site, as our initial ANOVA detected no differences among the various types of crosses. With our nine line means, we tested for six genetic composite effects that can explain the deviation of mean fitness from a population with random segregation and assorting of all alleles (F_∞ population), following Bieri and Kawecki (2003). Composite effects tested were: additive (a), dominance (d), additive × additive epistasis (aa), additive × dominance epistasis (ad), dominance × dominance epistasis (dd), and additive maternal effect (am). An additive effect would indicate a difference between the two species in additive effects of their genes. A dominance effect is the sum of effects of loci that carry alleles inherited from different parental populations (e.g., all loci in F1 and half of loci in F2; Table 4 in Results). The epistatic terms reflect interactions between pairs of loci. The additive maternal effect corresponds to effects of maternal genotype for the lines that have mothers belonging to one of the species.

Analysis proceeded by fitting linear models to the nine line means. Each model had the form:

$$\overline{Y_i} = m + x_a[a] + x_d[d] + \cdots + e_i,$$

where $\overline{Y_i}$ is the mean of line i, x_a, x_d etc. are the regression coefficients for the six genetic parameters for each of the nine line types (Table 4 in Results; Bieri and Kawecki 2003), and e_i is the residual error. Parameters were estimated by minimizing the sum of the weighted residual squares ($\mathrm{RSS_w}$), where the weight is the reciprocal sampling variance of the line mean (Lynch and Walsh 1998):

$$RSS_w = \sum_{i=1}^{9} \frac{e_i^2}{SE_i^2}.$$

We compared the fit of 16 possible models. Each model either included or excluded the additive parameter (a), the dominance parameter (d), the set of epistasis parameters (aa, ad, dd), and the maternal parameter (am). The model with the lowest Akaike's information criterion (AIC) was selected as most parsimonious. Assuming normally distributed data, $\mathrm{AIC} = RSS_w + 2K + \text{constant}$, where K is the number of parameters in the model and the constant is the same for all models. Once we obtained the most parsimonious model, we then evaluated the significance of each parameter using a likelihood-ratio test (Lynch and Walsh 1998):

$$\Lambda = RSS_{w(\text{reduced model})} - RSS_{w(\text{full model})}$$

which is chi-square distributed with degrees of freedom equal to the difference in number of parameters between the full and reduced models.

Table 1. Seeds per pollination for nine types of crosses in two years. *N*, number of maternal families. Maternal families with fewer than five pollinations were not included in the analysis.

Cross type	1997 seeds/ pollination mean±SE	*N*	1998 seeds/ pollination mean±SE	*N*
AA	11.8±1.44	9	6.1±1.28	9
AH	9.4±1.61	5	6.2±1.37	5
AT	8.6±1.53	5	6.7±1.17	5
HA	5.1±0.79	4	6.4±2.12	5
HH	8.9±1.08	5	7.3±1.22	5
HT	6.8±0.79	4	6.7±1.26	5
TA	6.4±2.50	5	4.4±1.45	5
TH	3.7±1.87	4	5.3±2.03	5
TT	4.9±1.08	10	8.3±1.35	10

Results

HYBRID FORMATION FROM CROSSES

All types of crosses consistently produced seeds (Table 1). In 1997, intraspecific *I. aggregata* crosses (AA) had higher mean seeds per pollination than did *I. tenuituba* crosses (TT) (a priori contrast $F_{1,42} = 16.90$, $P = 0.0002$). Crosses producing F1 hybrids had intermediate seed sets, with no statistical difference from conspecific crosses with the same maternal parent (AT vs. AA, $P = 0.1216$; TA vs. TT, $P = 0.4657$), nor between the reciprocals AT and TA ($P = 0.3448$). Crosses producing F2 hybrids (HH) had seed set intermediate between the two parental species and not significantly different from the average of the midparent and F1 hybrids ($P = 0.6182$). For the 1998 crosses, mean seeds per pollination did not differ significantly among the nine cross types (ANOVA $F_{8,45} = 0.59$, $P = 0.7802$), and none of the a priori contrasts were significant (all $P > 0.10$).

FITNESS AT THE HYBRID SITE

At the hybrid site, no significant differences were detected in lifetime fitness of progeny, as assessed by λ (Table 2; Fig. 2B). However, Ro, a measure of fitness not incorporating age at reproduction, showed both heterosis ($F_{1,37} = 4.83$, $P = 0.034$) and a difference between the two reciprocal F1 hybrids (i.e., AT vs. TA; $F_{1,37} = 10.04$, $P = 0.003$). Both of these patterns were due primarily to an unusually high mean value of Ro for TA hybrids (7.97)

Table 2. Fitness variation among nine types of maternal families planted at the hybrid site, analyzed with ANOVA and a priori contrasts. F1 vs. midparent: contrast of the average for AT and TA with the average for AA and TT. Hybrid breakdown: contrast of F2 with the average for AA, AT, TA, TT. For the composite measure of λ, the residuals were not normally distributed, and P values obtained with randomization tests are noted in parentheses.

Fitness measure	Source of variation	df	MS	*F*	*P*
Lifetime (λ)	Cross type	8	0.1548	0.40	0.914
	AA vs. TT	1	0.0027	0.01	0.934 (0.95)
	F1 vs. midparent	1	0.0158	0.04	0.841 (0.86)
	AT vs. TA	1	0.1926	0.50	0.486 (0.54)
	Hybrid breakdown	1	0.0852	0.22	0.642 (0.66)
	Error	37	0.3885		
Survival	Cross type	8	0.00050	0.64	0.736
	AA vs. TT	1	0.00068	0.86	0.358
	F1 vs. midparent	1	0.00001	0.02	0.894
	AT vs. TA	1	0.0019	2.48	0.124
	Hybrid breakdown	1	0.00002	0.03	0.860
	Error	37	0.00078		
Seeds per survivor	Cross type	8	5748.2	3.26	0.015
	AA vs. TT	1	1092.7	0.62	0.440
	F1 vs. midparent	1	21397.2	12.14	0.002
	AT vs. TA	1	4009.0	2.27	0.147
	Hybrid breakdown	1	12326.2	6.99	0.016
	Error	20	1762.6		
Age at reproduction	Cross type	8	2.9753	0.54	0.811
	AA vs. TT	1	4.2013	0.77	0.392
	F1 vs. midparent	1	0.0299	0.01	0.942
	AT vs. TA	1	10.0278	1.83	0.192
	Hybrid breakdown	1	0.7931	0.14	0.708
	Error	20	5.4895		

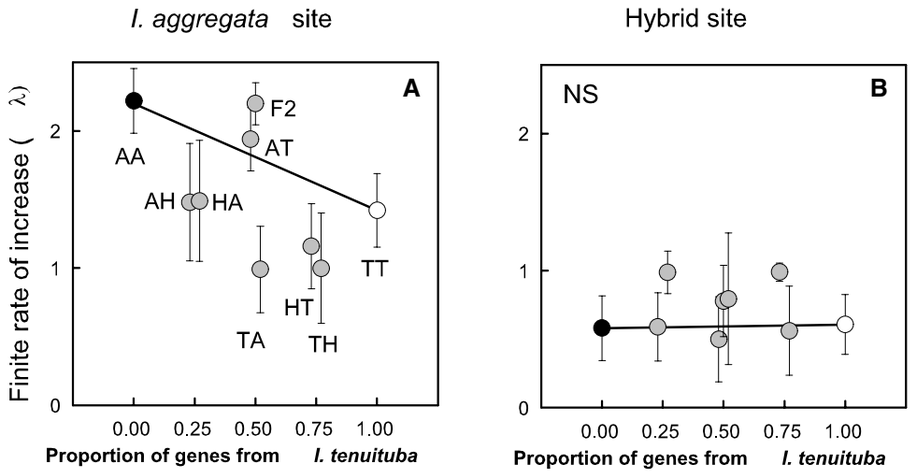

Figure 2. Finite rate of increase (λ) for seeds as a function of the proportion of genes from *I. tenuituba*. Means and standard errors across full-sibling families are shown. Closed circle: AA crosses. Open circle: TT crosses. Gray circles: Hybrid crosses as indicated. Symbols for hybrid crosses are slightly offset to allow viewing of multiple cross types with the same nuclear composition. Cross types are labeled where the overall ANOVA was significant ($P < 0.05$). (A) *I. aggregata* planting site. (B) Hybrid planting site.

compared to either AT hybrids or the parental species (means = 1.16, 1.73, and 1.48 for AT, AA, and TT, respectively). One fitness component, the number of seeds produced per survivor, showed heterosis, with AT and TA plants making more seeds than the parental species at this site (Fig. 3E; Table 2). F2 plants that survived to reproduction made fewer seeds per plant than the average for the F1 and midparent (Table 2), although they showed no reduction in lifetime fitness, measured either as Ro or λ (Fig. 3D; Table 2; $F_{1,37} = 1.15$, $P = 0.290$).

FITNESS AT THE *I. AGGREGATA* PARENTAL SITE

Plants had higher survival overall at the *I. aggregata* site, giving more power for detecting differences in reproductive success at that site. Plants of the home species, *I. aggregata*, achieved higher λ values than did plants of *I. tenuituba* (Fig. 2A; Table 3), reflecting, in part, their more than twofold chance of surviving to flower (mean = 0.101 vs. 0.037; Fig. 3A; $P = 0.002$). To assess the relative effects of cross type and maternal family in determining survival, we used Proc Glimmix in SAS to perform an additional analysis of survival for individual seeds (assumed to be binomially distributed), that included maternal family as a random factor nested within cross type. A likelihood-ratio test comparing the fit of this model to a model without maternal family found no significant variation at the family level. Instead survival differences were primarily at the level of cross types.

Plants of *I. aggregata* not only had higher survival at their home site, but also reproduced at an earlier age, approximately 3 years rather than 5 years for *I. tenuituba* ($P = 0.017$; Fig. 3C). Backcrosses to *I. tenuituba* (HT and TH) had the latest onset of reproduction and departed the most from the overall mean in this fitness component (Fig. 3C). Seeds per plant for those plants surviving to reproduce did not differ significantly among the cross types (Table 3). Dividing total seeds into two components, however, showed that, although the two species produced similar numbers of flowers, *I. aggregata* produced significantly more seeds per flower than did *I. tenuituba* (means = 2.20 and 1.25 across maternal families, contrast $F_{1,43} = 4.49$, $P = 0.041$ on log-transformed data). Ro followed the same pattern as λ, with AA seeds leaving an average of 13 seed descendants compared to six for TT seeds (contrast for log-transformed data; $F_{1,42} = 4.89$; one-tailed $P = 0.016$).

As observed in a previous study (Campbell and Waser 2007), lifetime fitness of F1 hybrids also depended on the direction of the cross, with AT hybrids achieving a λ nearly twice that of TA hybrids (one-tailed contrast $P = 0.025$), and also a higher Ro (one-tailed $P = 0.0196$). In second generation crosses (HA, AH, HT, TH, HH), the cytotype of the maternal parent did not significantly affect λ, but the trend was in the same direction as for the F1 generation. Only two of the 10 HA and HT families had *tenuituba* cytoplasm, so it was not possible to perform a specific test for cytonuclear interactions with these backcrosses. Overall there was no evidence for hybrid breakdown; fitness of F2 hybrids was not statistically distinguishable from the average for the parental species and F1 hybrids, and mean λ was actually higher (Fig. 2A). Fitness of progeny from crosses between natural hybrids (NN; mean λ = 1.52) did not depart significantly from that of F2 hybrids (HH; $F_{1,12} = 1.67$; $P = 0.22$).

Using line cross analysis of λ, the most parsimonious genetic model contained all effects except for the additive genetic effect ($RSS_w = 0.819$, df = 4; Table 4). When we removed the dominance effect, the set of epistatic effects, or the maternal effect one by one, all of the resulting models had significantly worse

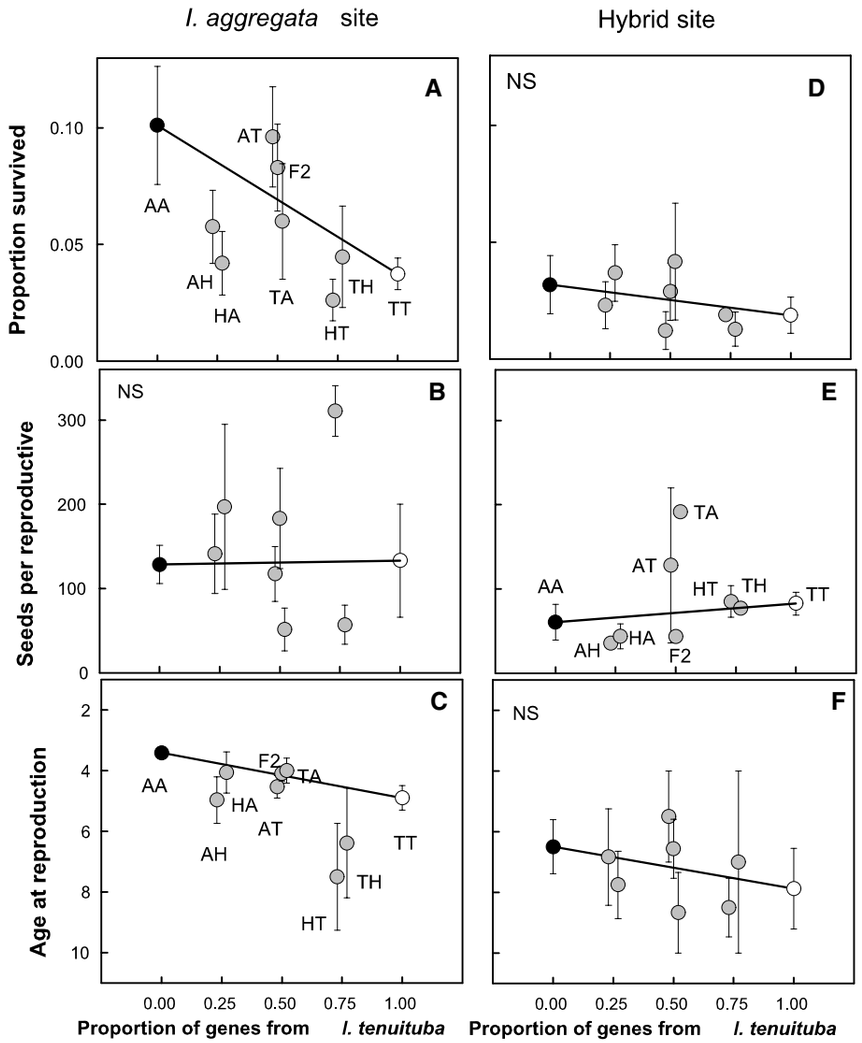

Figure 3. Fitness components for seeds as a function of the proportion of genes from *I. tenuituba*. Conventions as in Figure 2. (A) Proportion survived at *I. aggregata* site. (B) Seeds per reproductive individual at *I. aggregata* site. (C) Age at reproduction at *I. aggregata* site. (D) Proportion survived at hybrid site. (E) Seeds per reproductive individual at hybrid site. (F) Age at reproduction at hybrid site.

fit as judged by a likelihood-ratio test (all $P < 0.05$). The estimate for the maternal parameter was positive, reflecting higher fitness for lines with *I. aggregata* mothers. The negative parameter estimate for additive epistasis (aa) and positive estimate for dominance epistasis (dd) are both in the direction of higher fitness for hybrids than for the parental species (Table 4).

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F1 hybrids with *I. aggregata* as the mother (AT) had relatively higher fitness (λ) than the reciprocal hybrid (TA) at the *I. aggregata* site, as compared with the hybrid site, where the trend was actually in the opposite direction ($P = 0.028$). The relative fitnesses of the two species also differed in the expected direction between sites, with the relative performance of AA compared to TT much higher at the *I. aggregata* site ($P = 0.045$). No other contrasts specifying site × cross type interactions were significant (Table 5). The level of heterosis did not differ significantly between sites, and neither did the level of hybrid breakdown. Finally, the difference between backcrosses to *I. aggregata* (AH and HA) and backcrosses to *I. tenuituba* (TH and HT) in lifetime fitness did not differ significantly between the two sites.

Discussion

One of the most important results of this study is that F2 hybrids between these two species of *Ipomopsis* had female fitness just as high, if not higher, than expected under an additive model of inheritance. F2 seeds were readily produced with no reduction below that expected from the average for the parental species and F1 hybrids. Once formed, average lifetime fitness of the F2,

Table 3. Fitness variation among nine types of maternal families planted at the *I. aggregata* site, analyzed with ANOVA and a priori contrasts (conventions following Table 2). Lifetime fitness met the assumption of normally distributed residuals, whereas all of the fitness components had to be ln transformed.

Fitness measure	Source of variation	df	MS	*F*	*P*
Lifetime (λ)	Cross type	8	1.2711	2.28	0.034
	AA vs. TT	1	2.7521	4.94	0.016[1]
	F1 vs. midparent	1	0.7952	1.43	0.239
	AT vs. TA	1	2.2363	4.02	0.025[1]
	Hybrid breakdown	1	1.2898	2.32	0.135
	Error	42	0.5565		
Log (survival+1)	Cross type	8	0.0038	2.31	0.037
	AA vs. TT	1	0.0143	8.77	0.002[1]
	F1 vs. midparent	1	0.0005	0.28	0.570
	AT vs. TA	1	0.0029	1.77	0.095[1]
	Hybrid breakdown	1	0.0004	0.22	0.641
	Error	42	0.0016		
Log (seeds+1)	Cross type	8	2.1695	1.48	0.196
	AA vs. TT	1	2.2397	1.53	0.224
	F1 vs. midparent	1	0.7258	0.50	0.485
	AT vs. TA	1	3.4986	2.39	0.130
	Hybrid breakdown	1	2.7608	1.89	0.178
	Error	38	1.4625		
Log (age at reproduction)	Cross type	8	0.2214	2.85	0.014
	AA vs. TT	1	0.4827	6.22	0.017
	F1 vs. midparent	1	0.0103	0.13	0.718
	AT vs. TA	1	0.0364	0.47	0.497
	Hybrid breakdown	1	0.0004	0.01	0.942
	Error	38	0.0776		

[1]one-tailed test based on prediction made from prior results.

calculated as λ, was also statistically indistinguishable from expectation. Furthermore, for individuals planted at the *I. aggregata* site, additive × additive epistasis and dominance × dominance epistasis were actually in the direction of higher than expected fitness for the F2. There was no evidence for hybrid breakdown due to Dobzhansky–Muller incompatibilities between nuclear genes. Along with our finding of high fitness for crosses between natural hybrids, and earlier studies that also showed high fitness in

Table 4. Coefficients for genetic parameters and estimates of parameters for the finite rate of increase in the most parsimonious model at the *I. aggregata* site. $^{*}P < 0.05$. $^{**}P < 0.01$.

Line	Intercept	Parameter					
		a	d	aa	ad	dd	am
AA	1	1	0	1	0	0	1
TT	1	−1	0	1	0	0	−1
AT	1	0	1	0	0	1	1
TA	1	0	1	0	0	1	−1
F2	1	0	0.5	0	0	0.25	0
AH	1	0.5	0.5	0.25	0.25	0.25	1
HA	1	0.5	0.5	0.25	0.25	0.25	0
HT	1	−0.5	0.5	0.25	−0.25	0.25	0
TH	1	−0.5	0.5	0.25	−0.25	0.25	−1
Estimate	5.57**	—	−9.39**	−3.74**	0.07	5.31*	0.40**
SE	0.531	—	1.425	0.523	0.429	0.925	0.065

Table 5. Comparisons of lifetime fitness (λ) across two sites of planting crossed with nine types of maternal families, analyzed with a priori contrasts. BC_A, backcrosses to *aggregata* (AH and HA); BC_T, backcrosses to *tenuituba* (HT and TH).

Contrast	df	MS	*F*	*P*
Species × site	1	1.4131	2.96	0.045[1]
F1 vs. midparent × site	1	0.4975	1.04	0.311
AT vs. TA × site	1	1.810	3.79	0.028[1]
Hybrid breakdown × site	1	0.2965	0.62	0.433
BC_A vs. BC_T × site	1	0.3456	0.72	0.380
Error	79	0.4779		

[1]One-tailed test.

first-generation hybrids (Campbell and Waser 2001; 2007), these results run counter to any model of hybrid zone dynamics that relies solely on intrinsic nuclear genetic incompatibilities.

These results are based on female fitness. Whereas we were unable to measure lifetime male fitness of hybrids in these field environments, several male components of fitness have been measured in previous studies. Pollen production, siring success in single donor crosses, and dispersal of pollen to stigmas are all as high for F1 and F2 hybrids as for the parental species (Campbell et al. 2002, 2003). Pollen from F2 hybrids does show reduced siring success when placed on a stigma in competition with conspecific pollen, and this process could in principle contribute to hybrid breakdown through male function (Campbell et al. 2003). However, the effect is small because reproductive success of flowering hybrids relative to the parental species was similarly intermediate to the parental species whether based on seed production or on seeds sired (Meléndez-Ackerman and Campbell 1998).

Instead of evidence for intrinsic nuclear genetic incompatibilities, here we found evidence for environment-dependent selection. This selection was of two sorts. First, *I. aggregata* had higher fitness than *I. tenuituba* at its home site, but not at the hybrid site. Second, AT hybrids had higher fitness than TA hybrids at the *I. aggregata* site, even though the two reciprocal hybrids performed similarly at the hybrid site. Effects of the maternal parent were also evident in the line cross analysis at the *I. aggregata* site. These results are likely due to cytoplasmic (chloroplast or mitochondrial) genetic effects, or to interactions between nuclear genes and maternally inherited cytoplasmic genes (cytonuclear interactions), rather than to environmental maternal effects. Environmental maternal effects are often propagated via an effect of seed mass, but seed mass does not contribute to differences among cross types in survival in this hybrid zone (Campbell and Waser 2001), nor could it explain the differences among reciprocal hybrids that have been observed in the photosynthetic physiology of these species raised in pots (Wu and Campbell 2007). Furthermore, all seeds used in the study were generated in the same screenhouse environment. Asymmetry in the performance of offspring from a cross and its reciprocal has long been known in plants, and received sufficient attention by Darwin that Turelli and Moyle (2007) referred to it as Darwin's corollary. Cytonuclear interactions have been described in many other studies of plant hybrids (Burke et al. 1998; Tiffin et al. 2001; Fishman and Willis 2006; Etterson et al. 2007) as well as animal systems (Burton et al. 2006).

Our claims about environment-dependent selection require one caveat. Because seeds were planted into the two sites in two different years, we cannot be sure that the differences all relate to site per se. There are, however, two reasons that they are likely to reflect site differences. First, the two experiments mostly overlapped in time period, for nine of the total 10 years (1997–2006 vs. 1998–2006). Any difference due to time would have had to begin in the first year, between seed formation and emergence of a seedling. In previous studies we detected no differences among crosses in survival during that first year (Campbell and Waser 2001), that is, seedling emergence did not vary with cross type. And in the current study, none of our a priori contrasts were significant for survival to the first year at either site (all $P > 0.10$). Second, we saw the same patterns for relative fitness of the parental species (AA and TT crosses) and F1 hybrids (AT and TA crosses) in this study as we saw in an earlier experiment in which we did plant all seeds simultaneously into multiple sites (Campbell and Waser 2007). In both studies, lifetime fitness for AA exceeded that for TT at the *I. aggregata* site, but not at the hybrid site, and fitness for AT exceeded that for TA at the *I. aggregata* site, but not at the hybrid site. The major difference between this study and the earlier one is in the main effect of site, and not in interactions between site and type of cross. In the earlier study, plants had higher fitness overall at the hybrid site (Campbell and Waser 2007), whereas in the current study they had higher fitness overall at the *I. aggregata* site (mean $\lambda = 1.58$ vs. 0.68).

The absence of strong fitness differences at the hybrid site indicates a relaxation of selection, which may explain the preponderance of natural hybrids there. Based on molecular data showing that natural hybrids have primarily *I. tenuituba* cytoplasm, we proposed that the hybrid site was originally composed of plants of that species, and that *I. aggregata* alleles had introgressed through formation of F1s and repeated backcrossing (Wu and Campbell 2005). Our current data demonstrate that such backcrosses could be as successful at that site as pure *I. tenuituba*. At the *I. aggregata* site, backcrosses to the parental species are not similarly successful, but F1 hybrids with *I. aggregata* cytoplasm (AT hybrids) and F2 hybrids both have relatively high fitness. Given these patterns, it is not clear why natural hybrids of certain genotypes are not found at the *I. aggregata* site, raising the possibility that the area is in transition with an expanding hybrid zone. Pollen transfer patterns are unlikely to explain the apparent absence of hybrids. Although pollen is transferred more efficiently in the direction of

formation of AT hybrids, the asymmetry is slight (Campbell et al. 2002).

The higher fitness of *I. aggregata* plants at their home site reflected both higher survival to reproduction and more seeds produced per flower. The difference in seeds per flower was not as extreme as in our earlier study (Campbell and Waser 2007), which may reflect the appearance of substantial numbers of *Hyles lineata* hawkmoths in one year of the current study. Unlike hummingbirds, which are the most common flower visitors, these hawkmoths prefer plants with the narrower corolla tube characteristic of *I. tenuituba* (Campbell et al. 1997). The year they were present (2001) accounted for most of the very high seed sets for *I. tenuituba* at the *I. aggregata* site, with seeds per flower averaging 2.47 in 2001 vs. only 0.65 in the other years of blooming.

Several traits may help explain why F2 hybrids can have relatively high fitness. First, the F2 plants in this study have high photosynthetic water-use efficiency, with no evidence for hybrid breakdown in that trait (Campbell et al. 2005). Photosynthetic rate at optimal temperature also shows no hybrid breakdown, and photosynthetic rate at optimal light level is even higher for F2 hybrids than expected under an additive model (Wu and Campbell 2006). These physiological traits could contribute to high growth and/or survival, although that link has not yet been demonstrated in this particular hybrid zone. Once plants reach reproductive maturity, F1 and F2 hybrids also enjoy success at pollination (as estimated by dispersal of fluorescent dyes) equivalent to that of both of the parental species (Campbell et al. 2002).

In this *Ipomopsis* hybrid zone, cytoplasmic effects (either main effects or cytonuclear interactions) and genotype by environment interactions appear more important in lowering lifetime hybrid fitness than do intrinsic genomic incompatibilities between nuclear genes. These results support hybrid zone models that include environmentally dependent selection, and do not support those models that rely solely on intrinsic nuclear genetic incompatibilities. Because nuclear Dobzhansky–Muller incompatibilities theoretically accumulate as the square of the divergence between the species (Orr 1995), and these two species show low divergence (Wu and Campbell 2005), this result is not entirely unexpected. Whether this is a general pattern for plant species with low genetic divergence will have to await further transplant experiments with natural hybrid zones in which it is possible to assess lifetime fitness of different hybrid classes in different environments.

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