Evolutionary Dynamics of an *Ipomopsis* Hybrid Zone: Confronting Models with Lifetime Fitness Data

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ABSTRACT: Interspecific hybridization is a recurring aspect of the evolution of many plant and animal groups. The temporal dynamics of hybrid zones and the evolutionary consequences of hybridization should depend on fitness of parental and hybrid individuals expressed in different environments. We measured lifetime fitness, including survival and reproduction, of plants of *Ipomopsis aggregata*, *Ipomopsis tenuituba*, and their F1 hybrids, in experimental plantings in a natural hybrid zone. Fitness, measured as the finite rate of increase (λ), depended strongly on environment. Each parental species performed well in its home locale and poorly in the locale of the other species. Hybrids performed as well as parents overall but enjoyed their highest fitness in the hybrid site. Furthermore, F1 hybrids with *I. tenuituba* as maternal parent survived well only at the hybrid site, suggesting a cytonuclear × environment interaction. These results support an "environmental cline" model of hybrid zone dynamics, with complexities in the fitness of hybrids consistent also with an "evolutionary novelty" model. Combined with those of earlier studies of pollination, our results suggest that both vegetative adaptation to physical environment and floral adaptation to pollinators contribute to observed patterns of phenotypic expression in this hybrid zone and to persistence of the hybrid zone.

Keywords: age-specific fecundity and survival, environmental cline, evolutionary novelty, hybrid fitness, hybrid zone, *Ipomopsis*.

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Actual or potential hybridization between species is far from rare in some groups of plants and animals (Ellstrand et al. 1996; Arnold 1997; Rieseberg et al. 2006). Furthermore, past hybridization is implicated in the origin of numerous taxa, in particular many flowering plants (Rieseberg and Ellstrand 1993; McDade 1995; Rieseberg 1997). For these reasons, and also because hybridization may provide a window on the process of species formation (Barton and Hewitt 1989; Harrison 1990; Futuyma and Shapiro 1995; Arnold 1997), the study of hybridization and of natural hybrid zones has a long history in evolutionary biology.

Various conceptual models have been advanced to explain the fate of interspecific hybrids and the temporal dynamics of natural hybrid zones. These models fall into two categories, depending on whether fitness is independent of or dependent on the environment (table 1). One traditional viewpoint involving environment-independent fitness is that hybrid fitness is intrinsically low because of genetic incompatibilities (Dobzhansky 1970) but that this low hybrid fitness is balanced by gene flow between the parental species, generating a hybrid zone that is stable over time (tension zone model; Barton and Hewitt 1985). Genetic incompatibilities could be the result of interactions between nuclear genes or between cytoplasmic and nuclear genes (Burke et al. 1998). Other environment-independent scenarios assume that alleles differing between the parental species are neutral, so that the hybrid zone is expanding (neutral model) or that one species has higher fitness, in which case its traits can advance across the hybrid zone (advancing wave model; Fisher 1937). Alternatively, fitness might be environment dependent in such a way that each species has higher fitness in its own environment, producing clinal variation in genotypes and phenotypes across the boundary between parental environments (environmental cline model; Endler 1977) or a mosaic of patches of the two species (Harrison and Rand 1989), both of which are stable over time. Hybrids, in general, could be more fit than either of the parental species in particular habitats (bounded hybrid superiority model; Anderson 1948; Moore 1977), or hybrids of par-

Table 1: Some assumptions made by models for clinal hybrid zones and the coefficients for a priori contrasts used in testing the corresponding null hypotheses

Model	Fitness of parents and hybrids	Coefficients for contrast
Environment-independent models:[a]		
Advancing wave	One parent higher	AA − TT
Tension zone (converse is heterosis)	Hybrid lower than parents	.5AA − .5AT − .5TA + .5TT
Cytonuclear interaction	Hybrid fitness depends on direction	AT − TA
Neutral model	Equal	No significant effect of cross type
Environment-dependent models:[b]		
Environmental cline	Each parent has higher fitness in own habitat	.5AA@A − .5AA@T − .5TT@A + .5TT@T
Bounded hybrid superiority A	Hybrids higher than parents at hybrid site	−.5AA@H + .5AT@H + .5TA@H − .5TT@H
Bounded hybrid superiority B	Hybrids higher at hybrid than at parental sites	−.25AT@A + .5AT@H − .25AT@T −.25TA@A + .5TA@H − .25TA@T
Evolutionary novelty	Some hybrid genotypes higher than parents in some habitats	.25AT@A − .5AT@H + .25AT@T − .25TA@A + .5TA@H − .25TA@T[c]

Note: Assumptions of the environment-independent models were tested by contrasting specific types of crosses (AA, AT, TA, and TT, where the first letter denotes identity of maternal parent) in a two-way ANOVA that also included the factor of site. Assumptions of the environment-dependent models were tested by performing contrasts in a one-way ANOVA of all 12 combinations of cross type and site (designated by the third letter in notation such as AA@A, where A, H, and T stand, respectively, for the *Ipomopsis aggregata*, hybrid, and *Ipomopsis tenuituba* sites).

[a] No cross type × site interaction.

[b] Cross type × site interaction.

[c] For the particular case of cytonuclear × environment interaction (Campbell and Waser 2001).

ticular genetic backgrounds could outperform the parents in particular environments (evolutionary novelty model; Arnold 1997). Because these models were introduced over a long period by different authors, it is unsurprising that they are neither exhaustive of the possibilities nor mutually exclusive. Nonetheless, the models provide a useful working framework for classifying major patterns in the dynamics of hybrid zones. Determining which models apply should allow us to understand processes that produce patterns of morphological and genetic differentiation across hybrid zones and should also illuminate mechanisms that maintain species, since low hybrid fitness constitutes postzygotic reproductive isolation.

Because the different models of hybrid zones make different assumptions about fitness and its dependence on environment, an obvious way to proceed is to assess fitness of individuals of the parental species and their hybrids of known genetic background in different natural environments (Rieseberg and Carney 1998). Increasing interest in distinguishing among the models has led to a recent surge in such field tests, including some with flowering plants, which are particularly well suited to reciprocal transplant studies (Clausen et al. 1940; Langlet 1971; Levin and Schmidt 1985). Recent field tests have been conducted with *Artemisia* (Wang et al. 1997; Miglia et al. 2005), *Ipomopsis* (Campbell and Waser 2001), *Iris* (Emms and Arnold 1997), *Piriqueta* (Rhode and Cruzan 2005), and *Prunella* (Fritsche and Kaltz 2000).

To our knowledge, none of these tests began with seeds of known genetic background and followed survival and reproduction through the entire life history to estimate lifetime fitness. Not only are such measurements likely to be essential for accurately distinguishing among alternative models of hybrid zone dynamics, but they would also allow us to compare the extent to which low hybrid fitness results from poor habitat adaptation, as opposed to poor pollination (Chase and Raven 1975). Poor adaptation to soil types (Wang et al. 1999) or other aspects of the physical environment, as reflected in low survival, would be consistent with the idea that reproductively isolated species are maintained by habitat differences (Nosil et al. 2005). Conversely, poor pollination of hybrids would be consistent with the long-standing viewpoint that interactions with animal pollinators generate the divergent selection required for formation and maintenance of distinct plant species (Grant 1949). Examining the extent to which lifetime fitness differences between the parents and the hybrids are due to survival or reproduction and determining whether these patterns depend on the environment are useful steps in assessing the relative importance of these two mechanisms.

Here we report on a reciprocal transplant experiment involving a high-altitude hybrid zone between *Ipomopsis aggregata* and *Ipomopsis tenuituba* (Polemoniaceae) at Poverty Gulch, Gunnison County, Colorado. These herbaceous plants are semelparous (i.e., monocarpic), dying af-

ter a single bout of reproduction, making them amenable to estimates of lifetime fitness (Waser and Price 1989; Campbell 1997). In 1994, we initiated a reciprocal transplant study in which we placed parental and F1 hybrid seed into parental and hybrid habitats. We have now followed the offspring in the reciprocal transplant for 11 years to measure lifetime fitness, which allows us to address the following questions. (1) How does the lifetime fitness, measured as λ, the finite rate of increase, compare for the two parents and the two reciprocal F1 hybrids? (2) Do these fitness differences depend on the environment, and if so, which model of environment-dependent fitness do they support? (3) To what extent are the fitness differences due to viability or to reproductive differences, as assessed by elasticity and life table response analyses?

Methods

Natural History

Ipomopsis aggregata (Pursh) V. Grant (scarlet gilia) and *Ipomopsis tenuituba* (Rydb.) V. Grant are most likely each other's closest relatives (Grant and Wilken 1986; Wolf et al. 1993, 1997). For both species, seeds produced in a given summer germinate the following spring (further seed dormancy is rare), and by the end of the summer of seedling emergence, they have produced small basal rosettes of leaves. Individuals at our study sites in Colorado stay in that vegetative rosette stage for several years (2–10 years at 2,900-m elevation) before flowering and dying (Campbell 1997). After seed production in late summer, the parent plant dies at the first hard frost of the autumn. Flowers of *I. aggregata* ssp. *aggregata* have relatively short, wide, and red corolla tubes, while those of *I. tenuituba* ssp. *tenuituba* have relatively long, narrow, and pale-colored corolla tubes. At Poverty Gulch, the main pollinators of both species are hummingbirds, with hawkmoths also visiting in occasional years (two of the years between 1992 and 2003; Campbell 2004). The flowers are self-sterile because of late-acting incompatibility (Waser and Price 1991; Sage et al. 2006).

Experimental Plantings

Detailed methods for the design of our reciprocal transplant study are provided by Campbell and Waser (2001), so we give a briefer account here. At Poverty Gulch, plants of *I. aggregata* ssp. *aggregata* grow in the valley at elevations of 2,900 m and below, plants of *I. tenuituba* ssp. *tenuituba* on steep slopes above 3,100 m, and natural hybrids on open talus slopes at intermediate elevations (Grant and Wilken 1988; Wu and Campbell 2005). Despite their higher elevations, *I. tenuituba* and hybrid sites reach higher

maximum temperatures and lower relative humidities in summer than do the more heavily vegetated, flatter *I. aggregata* sites (Wu and Campbell 2006). In 1994, we collected parental plants of *I. aggregata* and *I. tenuituba* from opposite ends of the hybrid zone outside the range of natural hybridization (sites L and C, respectively, in Campbell et al. 1997) and brought them to the Rocky Mountain Biological Laboratory, where controlled crosses were performed in a screen house. We used hand-pollinations to cross 10 pairs of *I. aggregata* (AA crosses), 10 pairs of *I. tenuituba* (TT crosses), and 12 pairs including one plant of each species (total = 64 plants). These crosses yielded 10 independent full-sib families for each of the two parental species and 12 full-sib F1 families for each of the two cross directions, that is, with *I. aggregata* as the maternal parent (AT crosses) and with *I. tenuituba* as the maternal parent (TA crosses; fig. A1 in the online edition of the *American Naturalist*). In our analyses, we treated each of the 44 full-sib family groups at each site as the unit of replication.

The seed progeny were planted into five sites without removal of existing vegetation. Because there is no seed bank, and because we avoided sites within seed dispersal distance (1 m) of flowering *Ipomopsis* plants (Waser and Price 1983), we could ensure that seedlings emerging at the planting locations were the ones we planted. Seeds from all four cross types (AA, TT, AT, and TA) were reciprocally planted into the source sites for the parental species and into a site at the center of the hybrid zone (site I in Campbell et al. 1997). These three sites are hereafter referred to as "clinal sites" because they occur along the cline of the natural hybrid zone (site A for *I. aggregata*, H for hybrid, and T for *I. tenuituba* in fig. A1). As a control for possible fitness effects derived simply from planting individuals far from their natal sites, we also planted seeds from the conspecific AA and TT crosses into an alternate lower-elevation *I. aggregata* site (SR) and an alternate higher-elevation site (DP) in which the plants resemble *I. tenuituba* but may include some hybrids (see map of these alternate sites in Campbell and Waser 2001). At each site, seeds were arranged in 20 randomized blocks with one seed from each full-sib family group in each block (except in a few cases where crosses produced insufficient seed). We used a planting frame strung with fishing line, and nails for corner markers, to plant seeds at 10-cm intervals that we could relocate in subsequent years. In total, we planted 3,129 seeds from the 44 full-sib family groups (see Campbell and Waser 2001). Two families were omitted from analysis because ≤5 seeds were planted at each site, giving a total of 126 family-by-site combinations at the clinal sites.

Measurements of Survival, Fertility, and Floral Traits

In late June to early July of the next 11 years (1995–2005), we noted survival for each of the 3,129 individually planted offspring. Survivorship to year 1 was the proportion of seeds that germinated and emerged as seedlings in the summer after planting. To prevent escape of foreign genes into surrounding populations, approximately three times per week during most of the blooming season, we emasculated flowers on all those plants that bloomed by removing immature anthers with fine forceps. Fruits were collected every 2–3 days just before they would have dehisced, and seeds were counted. Fruits with frass from larvae of the seed predator fly *Delia* spp. (= *Hylemya* spp.; Brody 1992) inside usually had no intact seeds and, if so, were assigned zero reproductive success. A few fruits without a *Delia* exit hole had dehisced before collection, in which case their seed number was estimated as the mean for other successful fruits. Calyces are retained on the senescing plant, allowing us also to collect all flowers that failed to produce a fruit and hence produced no seeds.

For each of the full-sib families at each site, we determined (1) survival to reproduction, (2) mean seed production for blooming plants, (3) mean age at reproduction, and (4) the finite rate of increase (λ) as an estimate of lifetime fitness (Lande 1982; McGraw and Caswell 1996; Caswell 2001). Survival to reproduction was the proportion of plants reproducing out of the number of seeds planted. To estimate seed production by each plant, we multiplied total number of flowers (including those sampled for floral trait measurements; see below) by mean seeds per flower for that plant. In the first few years of the study, plants were caged near the end of the blooming season as an alternate means of preventing gene flow, requiring a more complex estimation of seed production (see Campbell and Waser 2001). Mean age at reproduction was the age averaged over blooming plants.

Age-specific survival (l_x) and reproduction (m_x) were used to calculate λ. We calculated λ as the dominant right eigenvalue for the Leslie projection matrix for each full-sib family at each site, using the program Populus for a birth pulse, postbreeding census, and checked the result with procedure IML in SAS (ver. 8.2; see "Elasticity and Life Table Responses"). In this formulation, the projection matrix contains survival from one year to the next ($P_i = l_i/l_{i-1}$) and fertility at each age i ($F_i = P_i m_i$). Use of a prebreeding formulation did not change λ. By the end of the study, 85 plants had flowered and then died, 74 of which successfully produced seeds, and 3,035 had died without ever producing flowers. At the end of the 2005 season, only nine plants remained alive in the vegetative stage (two in the highest-elevation clinal *I. tenuituba* site, six plants in the hybrid site, and one in the alternate *I.*

aggregata site). Unless noted below, estimates of λ assume that these plants will flower in year 12 with the mean seed production (including zeroes) for their family and site. In previous studies of *Ipomopsis*, most plants that lived past 8 years eventually bloomed (Campbell 1997). Terminating the study without full data from the final nine plants has minimal effect on conclusions, because elasticity to events in year 12 was extremely low.

Our use of λ as a fitness measure assumes that patterns of male reproductive output (seeds sired) are similar to patterns of seed production. We were unable to measure male fitness because we needed to prevent genetic contamination of natural populations by pollen export. However, other studies with this same system have suggested that patterns of male and female reproductive success, at the level of comparing parental species with hybrids, are similar (e.g., Meléndez-Ackerman and Campbell 1998). Another potential issue is that λ is sometimes seen as an appropriate fitness measure only for expanding populations (but see Lande 1982; Hogendyk and Estabrook 1984). The pooled estimate of λ based on mean values for l_x and m_x exceeded unity (see "Results"), even though the means included individuals transplanted out of their normal habitats. Furthermore, de Jong and Klinkhamer (2005) suggest that λ is appropriate for species showing density dependence in several life stages.

Several floral measurements were made on each flowering individual to determine whether species differences were retained in the experimental plantings (which can be viewed as common gardens), indicating a genetic basis to these differences. Of seven traits measured on naturally occurring *I. aggregata* and *I. tenuituba* at Poverty Gulch, five differ between the species (Campbell et al. 2002*a*; Campbell and Aldridge 2006). Nectar production was omitted because measuring it is time-consuming and often damages flowers, leaving us with corolla length, corolla width, minimum stamen length, and color as assessed by maximum optical density between 520 and 535 nm (see Campbell et al. 1997, 2002*a* for details). For each plant, we calculated mean values of these traits from samples averaging 4.4 flowers for morphological measurements and 2.6 corollas for optical density. We then averaged these values over plants in the same full-sib family at a particular site.

Statistical Analyses

To examine effects of cross type and site on λ, we used both standard general linear models and nonparametric randomization tests. We began by employing standard two-way ANOVA with fixed factors of cross type and site, which allowed direct comparison with analysis of the floral traits (see below). To test the assumptions of specific mod-

els for hybrid zones, we designed a priori contrasts (table 1). Unless otherwise indicated, all analyses used just the clinal data set with its three sites: parental *I. aggregata*, parental *I. tenuituba*, and hybrid (A, T, and H, respectively, in fig. A1). The sum of 3 df for the effect of the four cross types was subdivided into three orthogonal contrasts. The first compared the two parental species AA and TT. Higher fitness overall for one species would support the advancing-wave model. The second tested the F1 hybrid mean against the parental mean (mean of AT and TA vs. mean of AA and TT). Fitness for hybrids lower than the parental mean would support the tension zone model. The third compared AT and TA. This contrast was motivated by our preliminary finding after the first 5 years of the study that the AT hybrids had higher overall survival than the TA hybrids, suggesting a cytonuclear effect (interaction between effects of cytoplasmic genes inherited through the mother and nuclear genes inherited through the pollen parent). Maternal effects are a less likely explanation, in part because seed mass is similar for all parental and hybrid crosses (see also Campbell and Waser 2001). A cross type × site interaction would support an environment-dependent model of some sort.

To test the specific environment-dependent models (table 1), we also analyzed the 12 treatments (four cross types × three sites) as a one-way design (fig. A1). To test the environmental cline model, we asked whether the difference in λ for AA at the *I. aggregata* and *I. tenuituba* sites (hereafter designated AA@A vs. AA@T) equaled the difference for TT at the *I. aggregata* and *I. tenuituba* sites (TT@A vs. TT@T; table 1). We also analyzed this contrast for the full data set that included the two alternate sites in addition to the three clinal sites. To test the bounded hybrid superiority model, we contrasted the average fitness of F1 and the average fitness of the parent species specifically at the hybrid site (contrast a in table 1), and we also contrasted average F1 fitness at the two parental sites with average F1 fitness at the hybrid site (contrast b in table 1). The final contrast asks whether the effect of direction of hybridization (AT vs. TA) depends on the environment in such a way that AT performs relatively better than TA at the parental sites compared to at the hybrid site. Again, this contrast was motivated by finding such a pattern for 5-year survival (Campbell and Waser 2001), and it would be consistent with the evolutionary novelty model. Finally, the absence of any detectable fitness differences would be consistent with the neutral model.

Because λ was often 0 for an individual full-sib family and residuals were not always normally distributed, we used two-tailed randomization tests (Manly 1997) to test further the significance of specific contrasts (Caswell 2001) that were detectable from ANOVA. A macro in SAS permuted the values of λ at random among the 126 com-

binations of full-sib family and site and repeated this procedure 1,000 times to generate a null distribution of the contrast for comparison with the actual contrast.

Floral traits for the clinal data set were analyzed with the same two-way ANOVA we used to analyze λ. We found no site effect or cross type × site interaction, so we also performed one-way ANOVA and MANOVA, supplemented by a priori contrasts between the two species and between the means of the species and the F1 hybrids, to test for heterosis.

We used two general approaches to examine to what extent fitness differences were due to effects on (1) survival to reproduction, (2) mean fecundity of blooming individuals, or (3) mean age at reproduction. With generalized linear models, we first tested separately for effects on these fitness components and on seeds produced per seed planted (net reproductive rate $R_0 = a \times b$), log transformed, as an alternate measure of fitness that does not take into account age at reproduction. To maintain similar statistical power for all dependent variables, we used the same fixed-effects ANOVA models described above. For the dependent variable of survival, we also used procedure GENMOD (in SAS) with a binomial distribution to fit a factorial model with fixed effects of cross type and site. This particular analysis made an individual plant rather than a full-sib family the independent unit of replication, which seems justified, given that a mixed model, also including fixed effects of cross type and site, returned an estimate of 0 for the random effect of family nested within cross type (procedure GLIMMIX in SAS).

Elasticity and Life Table Responses

The above analyses tested for statistical significance of effects of cross type and site on various fitness components. However, they did not evaluate the quantitative contributions of survival and fertility to overall fitness. To do so, we employed matrix modeling to evaluate elasticities of λ and life table responses to the treatments.

Elastiticites describe how λ would respond to potential changes in age-specific survival and fertility by providing the response to a proportional perturbation in an element of the projection matrix. These were determined from standard matrix manipulations, as described by Caswell (2001) and implemented in SAS (using procedure IML to program the matrix operations). We determined elasticities to P_i and F_i, using a matrix calculated from the average values of age-specific survival (l_x) and reproductive output (m_x) over all treatments. Elasticity to F_i does not isolate the effect of reproduction on λ (because $F_i = P_i m_i$ includes survival from the preflowering year as well as reproduction), so we also determined elasticity to m_x by multiplying

Table 2: Effects of type of plant and site of planting along the cline on the finite rate of increase for a full-sib family, analyzed with ANOVA

Source of variation	df	MS	F	P
Analysis a:[a]				
Cross type	3	.779	1.99	.1188
Advancing wave	1	.153	.39	.5331
Tension zone	1	.125	.32	.5732
Cytonuclear	1	1.060	5.27	.0235
Site	2	2.556	6.68	.0018
Cross type × site	6	1.105	2.83	.0133
Error	114	.391		
Analysis b:[b]				
Environmental cline	1	2.844	7.28	.0080
Bounded hybrid contrast a	1	.614	1.57	.2128
Bounded hybrid contrast b	1	1.100	2.82	.0961
Evolutionary novelty	1	.710	1.82	.1804

Note: MS = mean squares.

[a] Two-way ANOVA with the effect of cross type of plant broken down into orthogonal a priori contrasts corresponding to environment-independent models as in table 1.

[b] Planned contrasts in one-way ANOVA corresponding to environment-dependent models as in table 1.

elasticity to F_i by the partial derivative of F_i with respect to m_x (Caswell 2001).

Elasticities do not necessarily describe how survival and fertility contributed to the actual effect of the treatments on λ, as these contributions also depend on how much the treatment affected particular life table events. We evaluated these contributions of survival and reproduction using demographic perturbation analysis (Caswell 2000). Following Caswell (2001), we determined the contributions of elements of the projection matrix to the treatment differences in λ by the element-by-element product of the matrix of differences multiplied by the sensitivity matrix evaluated at a point midway between the two matrices being compared.

Results

Lifetime Fitness

Averaged over sites, the two parental species had similar finite rates of increase λ (no support for the advancing-wave model), and the F1 hybrid mean did not differ from the parental mean (no support for the tension zone model, table 2, analysis *a*; fig. 1). However, hybrids with *Ipomopsis aggregata* as the mother (AT) overall outperformed the reciprocal cross (TA; a priori contrast $P < .05$; table 2, analysis *a*; $P = .036$ for randomization test), supporting a model that invokes a cytonuclear interaction. The two-way ANOVA revealed a cross type × site interaction in λ (table 2, analysis *a*), providing support for an environ-

ment-dependent model of fitness. *Ipomopsis aggregata* plants had higher fitness in their home site than in the *Ipomopsis tenuituba* site (mean $\lambda = 0.72$ vs. 0.09), and *I. tenuituba* plants had higher fitness in their home site than in the *I. aggregata* site (mean $\lambda = 0.44$ vs. 0.00). As a result, there was statistical support for an environmental cline model in which each species has relatively higher fitness at its home site (a priori contrast $P < .01$; table 2, analysis *b*; $P = .014$ for randomization test). The finite rate of increase was lower than replacement ($\lambda < 1$) for all 10 full-sib families of AA at the *I. tenuituba* site and all 10 families of TT at the *I. aggregata* site (in the latter case, fitness was 0 for all families). Fitness was similar for
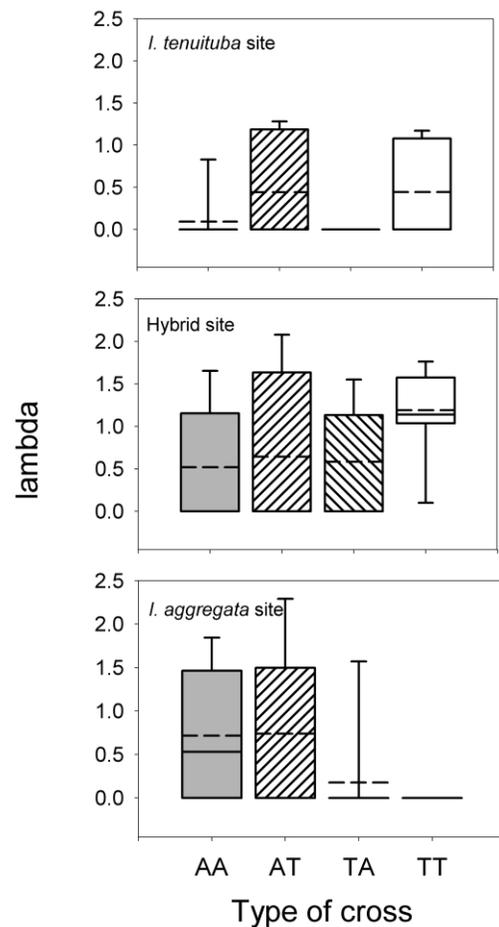

Figure 1: Box plots of the finite rate of increase (λ) of full-sib families for the four cross types planted as seed into the three clinal sites. The median is shown by a solid line; where not visible, the median equals 0. The seventy-fifth and twenty-fifth percentiles are indicated by the size of the box, and the ninety-fifth and fifth percentiles are shown by bars extending beyond the box. The mean value is depicted as a dashed line. All values were 0 for TA planted in the *Ipomopsis tenuituba* site and for TT planted in the *Ipomopsis aggregata* site.

F1 hybrids and the parental species at the hybrid site (little support for bounded hybrid superiority contrast a in table 2), although the F1 hybrids did tend to perform better in the hybrid site than they did in the parental sites (fig. 1; bounded hybrid superiority contrast b, $P < .10$; $P = .097$ for randomization test). This was primarily due to TA hybrids having relatively high fitness only in the hybrid site (fig. 1). No TA plant successfully reproduced at the *I. tenuituba* site. Number of seeds produced per seed planted (R_0) for each full-sib family showed patterns similar to those for λ. The F1 hybrids with *I. aggregata* as the mother performed better than the reciprocal cross, AA and TT plants each performed better in their home site than in the site of the other species, and hybrids performed better at the hybrid site than at the parental site (table 3).

Analysis of the two parental cross types at all five sites (data set including the alternate sites DP and SR) was also consistent with environment-dependent fitness. Two-way ANOVA revealed a species × site interaction (table A1 in the online edition of the *American Naturalist*). A priori contrasts showed that *I. aggregata* plants had higher λ at the two *I. aggregata* sites than at the *I. tenuituba* sites ($P = .0011$) and performed relatively better there than did *I. tenuituba* (environmental cline contrast comparing the difference between the species at the two *I. aggregata* sites and the difference at the two *I. tenuituba* sites, $P = .0061$).

Floral Traits

We observed no effects of site (all $P > .10$) and no site × cross type interactions (all $P > .25$) in two-way ANOVA for any of the floral traits, indicating that all trait differences were maintained in the common gardens. In one-way ANOVAs of the clinal data set, corolla length, corolla width, optical density, and minimum stamen length all retained differences between the two species (fig. A2 in the online edition of the *American Naturalist*). The F1 hybrids were intermediate between the parents for all four traits, although values for optical density (our measure of color; see Campbell et al. 1997, 2002*a*) were significantly lower than the parental mean (fig. A2). Multivariate analysis including all four traits confirmed differences in floral traits among the four cross types (Wilks's λ = 0.1029, $F = 6.94$, df = 12, 61, $P < .0001$), with the first canonical discriminant function correlating most strongly with optical density ($r = 0.90$) and corolla width ($r = 0.62$).

Table 3: Components of fitness as a function of type of cross (AA, AT, TA, and TT, with first letter denoting identity of maternal parent) and site of planting

	Clinal planting sites			Alternate planting sites	
Type of cross	*Ipomopsis aggregata*	Hybrid	*Ipomopsis tenuituba*	*I. aggregata* (SR)	*I. tenuituba* (DP)
Survival to reproduction (%):					
AA	3.02	2.77	.34	2.40	4.26
AT	5.21	4.51	2.06	…	…
TA	1.82	4.19	0	…	…
TT	.88	7.78	2.91	1.11	6.25
Mean fecundity of reproductives (seeds):					
AA	118.0	163.2	18.0	230.2	90.6
AT	158.0	567.0	89.2	…	…
TA	146.0	89.8	…	…	…
TT	0	129.2	40.5	6.0	73.8
Mean age at reproduction (years):					
AA	5.9	7.4	10.0	6.2	4.6
AT	6.0	6.5	8.5	…	…
TA	5.0	6.5	…	…	…
TT	5.0	7.5	7.5	10.0	4.9
Seeds produced per seed planted:					
AA	1.56	1.03	.05	1.78	2.09
AT	1.99	2.92	.90	…	…
TA	.36	1.44	0	…	…
TT	0	4.91	.65	.05	2.11

Note: Values were first averaged for all offspring from a full-sib family and then averaged over type of cross and site. Seeds produced per seed planted was estimated by back transforming the mean of log (survival × fecundity).

Fitness Components

In the clinal data set, two-way ANOVA revealed no cross type × site interaction for survival to reproduction ($P = .19$), fecundity of reproductives ($P = .60$), or mean age at reproduction ($P = .66$). Furthermore, the a priori environmental cline contrast was not significant for any of these fitness components ($P = .12$, .73, and .49, respectively). However, further analysis of survival assuming a binomial error distribution revealed significant effects of cross type, site, and their interaction (procedure GEN-MOD; table A2 in the online edition of the *American Naturalist*). A priori contrasts within the cross type factor showed higher survival to reproduction for AT than for the reciprocal TA type of F1 (likelihood ratio test, $P = .0033$; table A2) but no detectable difference between mean survival for hybrids and parental species ($P = .3570$). As for lifetime fitness (table 2), survival was lower at the high-elevation *I. tenuituba* site than at the other sites (table 3; $P = .0040$ for site effect in two-way ANOVA, and $P < .0001$ in procedure GENMOD), and mean age at reproduction was higher (table 3; $P = .0028$). In analysis of the parental crosses at all five sites, each species did have relatively higher survival at its own type of site (environmental cline contrast, $P = .0214$), but the ANOVA approach revealed no significant patterns in fecundity.

Life Table Analysis

Results were generally consistent with the ANOVA approach to analysis of fitness components, in emphasizing the importance of survival to reproductive age. Averaging over the treatment combinations of cross type and site, mean survival from seed to a vegetative rosette of age 1 was 25.4%. Subsequent survival of rosettes ranged from 33.9% for l_{11}/l_{10} to 86.3% for l_5/l_4 (table A3 in the online edition of the *American Naturalist*). Reproductive output (m_x) peaked at a value of 47.7 seeds at age 6. The projection matrix based on these average values had a finite rate of increase exceeding replacement ($\lambda = 1.249$, or 1.239, if the unknown reproductive rate in year 12 is set to 0 instead of its estimated value). Both sensitivities and elasticities of λ were generally higher for survival than for per capita fertilities or reproductive rates (fig. 2). Elasticity to survival, the response of λ to a proportional change, decreased with age once the earliest age of reproduction was reached. Elasticity to fertility peaked for individuals in the age class 6, which had the highest F_i in the projection matrix. Summed elasticity to survival over all age classes accounted for 83% of the total, versus 17% for elasticity to fertility (fig. 2).

As explained above, the clearest effects of cross type or cross type × site interactions on λ were higher fitness for
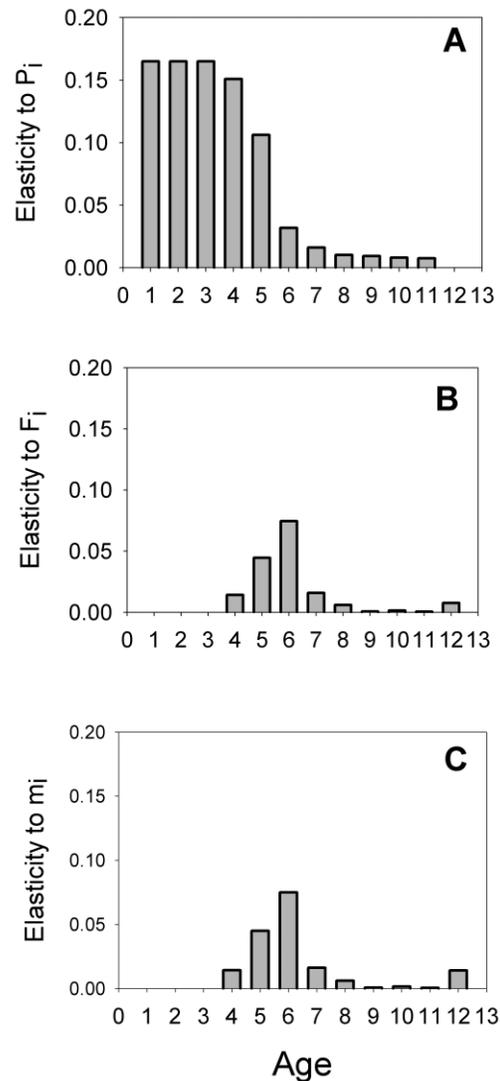

Figure 2: Elasticities of λ to (*A*) survival from one year to the next (P_i), (*B*) fertility (F_i), and (*C*) reproductive output (m_i) as a function of age class. The population projection matrix employed the average age-specific survival and reproductive output across 12 combinations of cross type and site.

I. aggregata plants at their home site, higher fitness for *I. tenuituba* plants at their home site, and higher average fitness for AT hybrids than for the reciprocal cross, TA. We thus focused on the life table responses to these effects. The single largest contribution to each of these treatment effects on λ was always fertility ($F_i = P_i m_i$) for individuals in one of the earliest reproductive age classes (fig. 3, *middle row*). Reproductive output for that age (m_i) also always had a larger contribution than any single age-specific survival (fig. 3, *bottom row* vs. *top row*). For example, AA began reproducing at the *I. aggregata* site in year 4 and
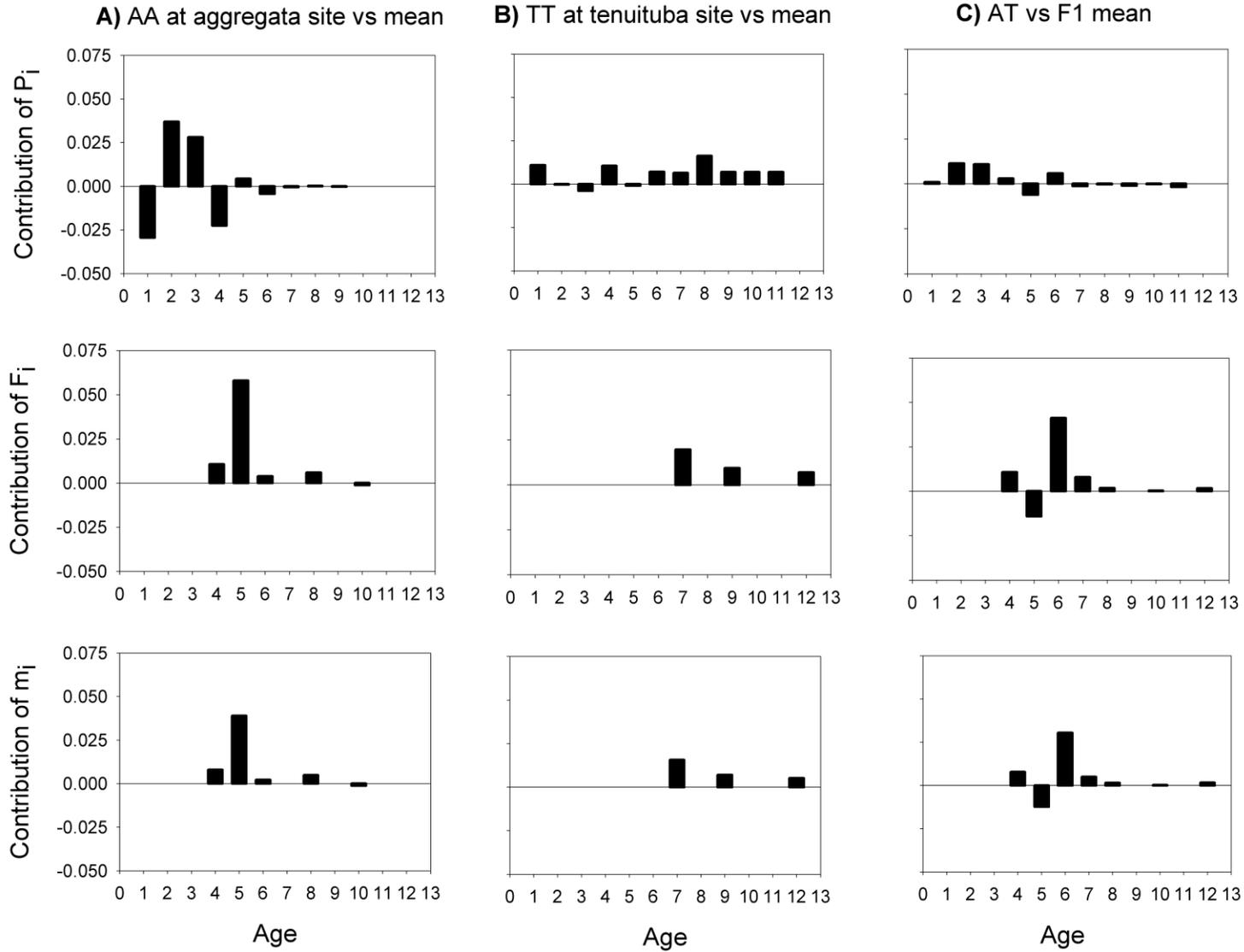
Figure 3: Contributions of survival from one year to the next (P_i), fertility (F_i), and reproductive output (m_i) to the effect of treatments on λ. *A*, Comparison of AA at the *Ipomopsis aggregata* site with its mean at the two parental sites. *B*, Comparison of TT at the *Ipomopsis tenuituba* site with its mean at the two parental sites. *C*, Comparison of AT with the mean for F1 hybrids.

reached its highest reproductive output in year 5 while producing no offspring in those years at the *I. tenuituba* site. That difference led to the largest contribution of fertility in year 5 to the difference between AA at its home site and the mean value at both parental sites (fig. 3*A*). Reproductive output in year 5 (m_5) also had a larger contribution than any single survival component. This pattern of the highest value for one of the fertility contributions occurred even though elasticity of the overall λ was always greater for survival (fig. 2), and the ANOVA approach detected no statistically significant effects on reproductive output. Comparison of TT at its home site with the mean for the parental sites (fig. 3*B*) revealed continuing contributions of survival across nearly all age classes, in contrast to other treatment combinations, where only survival up to age 5 or 6 contributed. However, the lifetime fitness of 0 for TT at the clinal *I. aggregata* site is the result of the failure of flowering individuals to make any seeds. In summary, both age-specific reproduction and survival contributed to the effects of cross type and cross type × site interactions on fitness (fig. 3).

Discussion

This study provides a comprehensive examination of how lifetime fitness of F1 hybrids between naturally hybridizing plants compares with that of the parental species in both parental and hybrid environments. The strongest conclusion is that fitness of the parental species and the hybrids depended on the environment in which they were planted. This result supports an environment-dependent model of hybrid zone dynamics over any environment-independent model. Furthermore, none of the specific predictions of environment-independent models were borne out. The F1 hybrids were equally as fit overall as the two parental species, which runs counter to the prediction of the tension zone model that hybrids are of low fitness but are constantly regenerated via gene flow between the parental species. This interpretation, however, is limited by our use of F1 hybrids only, as heterosis in the F1 compared to parental inbred lines might obscure hybrid breakdown due to epistasis that would be observed in later generations (Lynch and Walsh 1998). The neutral model, which posits no fitness differences, is also rejected because of the clear differences we detected. The advancing-wave model, which assumes that one parent has higher fitness, is also not supported. No one species performed the best in all habitats when we take into account both survival and reproduction. An earlier study showed that *Ipomopsis aggregata* had a clear advantage in pollinator visitation across all habitats at this same hybrid zone (Campbell et al. 1997), but survival of *Ipomopsis tenuituba* in this study was suf-

ficiently high to erase that reproductive advantage, so that *I. aggregata* did not have higher fitness overall.

The environmental cline model was the most strongly supported model. Each species of *Ipomopsis* performed better than the other in its own habitat, and F1 hybrids tended to perform better in the hybrid habitat than in the parental habitat. However, the better performance of hybrids in the hybrid habitat was not sufficient to elevate their fitness, as measured by the finite rate of increase, above that of the parental species at that site, which is an important prediction of the bounded hybrid superiority model. Overall, the bounded hybrid superiority model had weak support. We did observe strong effects of the direction of hybridization, with F1 hybrids having *I. aggregata* as the mother showing higher λ averaged over all three sites because of their better performance at both of the parental sites but not the hybrid site. As cytoplasmic genes (chloroplast and mitochondrial DNA) are inherited through the mother in these plants (Wolf et al. 1997), this last result suggests cytonuclear interactions for fitness, first proposed on the basis of survival by Campbell and Waser (2001). This provides some support for the evolutionary novelty model, which predicts that hybrid fitness will vary according to the specific genotype. However, the relative success of the two reciprocal F1 hybrids did not vary significantly between the parental and hybrid sites. Overall, our finding of environment-dependent fitness in this hybrid zone is consistent with those of most other reciprocal transplant studies with plant hybrids that relied on partial fitness measures (Emms and Arnold 1997; Fritsche and Kaltz 2000; Miglia et al. 2005).

The lower fitness for certain hybrids (TA) compared to the parental species in each of the parental sites is consistent with the idea that environment-dependent selection plays a strong role in speciation. Ecological speciation, as defined by Schluter (2000), predicts that hybrids have low fitness specifically in the parental sites as a result of poor adaptation to those environmental conditions. Since both types of reciprocal F1 hybrids had fitness approximately as high as the mean for the parental species at the hybrid site, we can infer that the low fitness in the parental sites involves environment-dependent selection rather than universal hybrid inferiority. Moreover, because the low fitness of TA hybrids resulted primarily from low survival, it likely reflects poor habitat adaptation. This type of selection not only could help to maintain the current cline but may also have been important in the original speciation event, because these two species diverged very recently (Wolf et al. 1993).

Our ability to measure fitness over the entire life span of individuals was critical to these conclusions. Based on comparison of elasticities, λ was more sensitive to potential changes in survival than to those in reproduction. At first

glance, this result might suggest that survival of hybrids is more critical to measure than reproduction. But the contributions of these two life stages also depend on the magnitude of the treatment differences in survival and reproduction. Both survival and reproductive output during the first big year of flowering made large contributions to differences in the finite rate of increase. The sources of mortality seen in this experiment (including drought, overgrowth, and herbivory by small mammals) were likely similar to those experienced by unmanipulated plants, although seedling emergence may have been higher than when seeds are dispersed naturally (15%; M. Price, D. Campbell, N. Waser, and A. Brody, unpublished data). On the basis of survival alone (Campbell and Waser 2001), we could not have predicted that *I. tenuituba* would have zero fitness at the *I. aggregata* site. On the basis of events during reproduction, including pollination (Campbell et al. 1997, 2002*a*) and predispersal seed predation (Campbell et al. 2002*b*), we could not have predicted the poor success of TA hybrids in the parental sites or the poor success of *I. aggregata* specifically at the *I. tenuituba* site. These findings illustrate how important it is to evaluate lifetime fitness in assessing the dynamics of hybrid zones. We are fortunate to study a system in which this is possible and encourage others to study plant hybrids with this feature.

Our results, in combination with those of earlier studies, suggest that both physiological adaptations that influence survival and pollinator adaptations that influence reproduction are important to the fitness patterns in this hybrid zone. Plants of *I. tenuituba* and F1 hybrids have higher water use efficiency (carbon assimilated through photosynthesis per water lost through transpiration) and a higher optimal temperature for photosynthesis than do plants of *I. aggregata* (Campbell et al. 2005; Wu and Campbell 2006). These physiological traits appear to match the higher maximum temperatures and vapor pressure deficits measured in the *I. tenuituba* and hybrid sites compared to those in the *I. aggregata* site. They thus provide a potential explanation for why *I. aggregata* has low survival in the *I. tenuituba* site, although a definitive test would require measuring the relationship of fitness to the physiological traits in a reciprocal transplant (and the higher survival of AT than TA hybrids at the parental sites remains a mystery). In turn, the low fitness of *I. tenuituba* in the low-elevation *I. aggregata* site is a consequence, in part, of low reproductive output. Three plants of *I. tenuituba* survived to bloom at the two *I. aggregata* sites and produced typical floral displays, but two of the plants produced no seeds, and one plant produced only six seeds, probably because hummingbirds discriminate against flowers of this species and in favor of *I. aggregata* and hybrids (Campbell et al. 2002*a*). Hawkmoths were not observed in the year that these particular plants flowered

(Campbell et al. 2002*a*). Species differences in floral traits were maintained in the common gardens, indicating a genetic basis to those differences, and hybrids were intermediate for all traits, as also observed for natural hybrids (Campbell et al. 1997). Differences in seed production among genotypic classes are likely not due to postpollination events or seed abortion, because hand pollinations between and within the two species and F1 hybrids produce similar seed sets (Campbell et al. 2003), with no evidence for any conspecific pollen advantage (Alarcón and Campbell 2000).

The fitness patterns allow us to make some predictions about the evolutionary fate of this hybrid zone. Because seed dispersal is so limited (typically <1 m; Waser and Price 1983), gene flow into each site is most likely dominated by pollen dispersal (Campbell 1991). At the *I. tenuituba* site, pollen with nuclear alleles of *I. aggregata* would be unlikely to lead to realized gene flow because F1 hybrids with *I. tenuituba* as the mother had zero survival. This suggests that the purity of *I. tenuituba* at high-elevation sites will be maintained. At the *I. aggregata* site, F1 hybrids with *I. aggregata* as the mother (AT) had fitness as high as the parental species. In this case, we do expect realized gene flow through pollen into the site. Hummingbirds are the main pollinators in this hybrid zone (Campbell et al. 1997) and are more efficient on a per-visit basis at transferring pollen from *I. aggregata* to stigmas of *I. tenuituba* than in the reverse direction (Campbell et al. 1998). This asymmetry could help to reduce formation of AT hybrids, although the reduction is expected to be slight after the frequency of pollinator flights in the two directions is taken into account (Campbell et al. 2002*a*). Alternatively, the hybrid zone could be out of equilibrium, in which case we may witness introgression of *I. tenuituba* alleles into the *I. aggregata* population in the near future. At the hybrid site, both types of F1 appear successful. It is striking, therefore, that Wu and Campbell (2005) found that nearly all natural hybrids have *I. tenuituba* cytoplasmic DNA markers. They proposed a nonequilibrium scenario in which the natural hybrid site represents a population that was historically composed of *I. tenuituba*, with the appearance of hybrids due to introgression of *I. aggregata* nuclear alleles through pollen movement and repeated backcrossing. Future measurements of fitness for F2 and backcross hybrids will help to refine these predictions.

In conclusion, we found strong evidence for environment-dependent selection in structuring this *Ipomopsis* hybrid zone. We stress that other contact zones between *I. aggregata* and *I. tenuituba* and between other pairs of species in this genus can have different spatial configurations and details of fitnesses (e.g., Wolf et al. 2001; Aldridge 2005) and perhaps quite different evolutionary dynamics, although this possibility remains to be explored. However,

the fact that parental and hybrid fitnesses in our case varied across habitats, with each type of individual, including hybrids, succeeding in a particular part of the clinal hybrid zone, lends further credence to the emerging view of hybrids as a stable feature in the diversity of flowering plants and of hybridization as a critical element of their macroevolution (Harrison 1990; Arnold 1997).

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