American Journal of Botany 93(6): 903–909. 2006.

ASYMMETRICAL POLLEN SUCCESS IN *IPOMOPSIS* (POLEMONIACEAE) CONTACT SITES[1]

GEORGE ALDRIDGE[2,4] AND DIANE R. CAMPBELL[2,3]

[2]Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, Colorado 81224 USA; and [3]Department of Ecology and Evolutionary Biology, University of California, Irvine, 321 Steinhaus Hall, University of California, Irvine, California 92697 USA

Variation in hybridization rates among contact sites of a species pair provides an opportunity for assessing the importance of individual reproductive isolating mechanisms in limiting gene flow between species and thus promoting speciation. Conspecific pollen advantage is common in angiosperms, but its importance as a reproductive isolating mechanism is uncertain. We compared the strength of conspecific pollen advantage in two *Ipomopsis aggregata–I. tenuituba* (Polemoniaceae) contact sites that differ in frequency of natural hybrids. We performed hand pollinations of single- and 1:1 mixed-species pollen loads, using donor and recipient plants from both contact sites. Paternity of offspring from mixed-species pollinations was determined using an allozyme marker. Donors from the high frequency hybrid site showed no conspecific pollen advantage; both species sired seeds in proportion to their fraction of the pollen load (0.5). In contrast, *I. aggregata* from the low frequency hybrid site sired 70–85% of offspring on recipients from both sites. These results suggest that pollen interactions can influence the level of natural hybridization. They also suggest the importance of geographic variation in reproductive isolation, which should be considered in studies of biological invasions and exposure of engineered crops to wild relatives.

Key words: Colorado; hybrid zones; *Ipomopsis*; Polemoniaceae; pollen competition; reproductive isolation.

Natural hybrid zones provide insights into the evolution of reproductive isolation, a critical step in the formation of new species (Hewitt, 1988; Rieseberg and Carney, 1998). Hybrid zones allow us to study not only how reproductive isolation arises, but also the range of possible evolutionary outcomes that follow its breakdown. The extent of hybridization can be very important to the conservation of rare species (Levin et al., 1996), control of biological invasions, and the management of genetically engineered traits in domesticated crops (Ellstrand et al., 1999). The breakdown of reproductive isolation can lead to the creation of genetic novelty (Arnold, 1997) and increased biodiversity, or it can result in the extinction or genetic swamping of populations and a concomitant loss of biodiversity (Mooney and Cleland, 2001). As impacts on the world's biota increase, it becomes increasingly urgent to understand the potential causes and evolutionary outcomes of changes in reproductive isolation. One approach involves comparing sites of contact between species that differ in reproductive isolation.

Where reproductive isolation is strong, a contact site between the species will typically have few intermediate individuals relative to the parental types (Harrison and Bogdanowicz, 1997). If reproductive isolation is instead weak, a contact site may include abundant intermediates. Contact sites of different species pairs exhibit a wide range of frequencies of intermediate individuals (Barton and Hewitt, 1985; Jiggins and Mallett, 2000), suggesting considerable variation in reproductive isolation between species. Differences among contact sites in the frequency of hybrids might also reflect differences in the lengths of time in which the species have been in contact in the respective sites or over which they have been diverging. The strength of reproductive isolating mechanisms thus must be tested using experimental manipulations that simulate equal opportunity for interspecific mating.

Many contact sites dominated by parental types lack endogenous postzygotic isolation, suggesting that exogenous isolation and/or prezygotic isolation are primarily responsible for determining the frequency of hybrids in nature (Jiggins and Mallett, 2000). However, this conclusion is based on comparisons of many contact sites between different pairs of species. Such comparisons could be confounded by between-taxon variation in evolutionary history (Coyne and Orr, 2004) or ecology. Variation in frequency of hybrids among contact sites of a pair of species would provide the opportunity to compare reproductive isolating mechanisms in contact sites while controlling for differences in evolutionary history (Williams et al., 2001). Several such systems are now known in flowering plants (Williams et al., 2001; Watano et al., 2004; Aldridge, 2005). This sort of variation allows us to test the hypothesis that if a given reproductive isolating mechanism is important in limiting gene flow, it should be stronger in a contact site where intermediate individuals are scarce.

As reproductive isolating mechanisms act sequentially, each can reduce only gene flow not prevented by earlier-acting mechanisms (Ramsey et al., 2003). However, by studying the same pair of species in conditions of relatively limited and relatively unlimited gene flow, we can focus on the forms of isolation that reduce gene flow from the amount present in the contact site where intermediate individuals are abundant. We can thereby quantify the importance of a particular isolating mechanism in structuring contact sites, even if an earlier-acting mechanism partially isolates the species throughout their ranges.

[1] Manuscript received 15 April 2005; revision accepted 6 March 2006.

This research was funded by NSF-DEB 44087-20690 to D.C. and G.A., NSF-DEB 9806547 to D.C., Sigma Xi GIAR and the Lee R. G. Snyder Memorial Fund at RMBL to G.A. Research within Black Canyon of the Gunnison National Park was performed under permit BLCA-2001-SCI-0001, and the authors thank Ken Stahlnecker of the National Park Service for his assistance in the permitting process. The manuscript was improved by comments from C. Wu, S. Kimball, N. Pohl, A. Weis, and two anonymous reviewers.

[4] Author for correspondence (e-mail: galdridg@uci.edu), phone: 970 349-5336, fax: 970 349-7481.

In flowering plants, prezygotic isolation can take several forms. Pollen might not be deposited on heterospecific stigmas due to behavior of pollinators, or it might fail to fertilize heterospecific ovules. Even when interspecific pollen transfer occurs naturally and species are fully interfertile, the success of heterospecific (Carney et al., 1996; Williams et al., 1999; Song et al., 2002) pollen at siring seeds might be reduced in the presence of conspecific pollen. This relatively poor performance of heterospecific pollen in competition with conspecific pollen is known as conspecific pollen advantage. Seeds from the same fruit often have different sires in natural populations, suggesting that mixed pollinations, and hence opportunities for pollen competition, might be common (reviewed in Bernasconi, 2004). Indeed, conspecific pollen advantage has been cited as an important cause of reproductive isolation (Arnold, 1997; Howard, 1999), not only in wild species but also between crops and wild relatives (Hauser et al., 1997). It can result from differences in pollen germination and growth rate, selective attrition of pollen tubes, or selective abortion of hybrid zygotes (Cruzan and Barrett, 1996). Pollen fitness differences are often associated with higher pollen tube growth rates in plants with longer styles (Emms et al., 1996). While several studies have demonstrated competition between pollen from different species, it is still unclear how important this phenomenon is in determining the rate of hybrid formation in nature. If it is a major determinant of hybrid formation, then pollen competition should be stronger in contact sites with relatively few hybrids.

We test this prediction that pollen competition is an important factor in limiting gene flow between plant species by comparing the strength of pollen competition in two contact sites of a species pair. Contact sites of *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae) and *I. tenuituba* (Rydb.) V. Grant vary in frequency of hybrids (Aldridge, 2005), and pollen competition in one contact site between them has been studied previously (Alarcón and Campbell, 2000). Using an allozyme marker to determine seed paternity, we asked if conspecific pollen advantage was stronger at a site where intermediate individuals are rare vs. a site where intermediates are abundant, and if pollen from one site showed similar patterns of competitive success on recipients from both sites. Conspecific seed siring success was measured as a disproportionate siring of seeds by the conspecific donor compared to the fraction of its pollen in mixed-species pollen loads.

MATERIALS AND METHODS

Study system—*Ipomopsis aggregata* subsp. *aggregata* and *I. tenuituba* are closely related species of montane wildflowers common in the western United States (Wolf et al., 1993; Grant and Wilken, 1988). These species have been treated as an example of premating isolation due to floral adaptation to hummingbird (*I. aggregata*) and hawkmoth (*I. tenuituba*) pollinators (Grant and Grant, 1965). Although these species hybridize readily (Grant and Wilken, 1988), contact sites differ in spatial structure and the frequency of hybrids (Aldridge, 2005). Two of these sites in western Colorado (CO) provide a contrast between a mosaic site with few intermediates and a clinal site where hybrids are common. Grizzly Ridge (GR), Montrose County, CO, lies on the north rim of the Black Canyon of the Gunnison between 2375 and 2438 m a.s.l. Both *I. aggregata* and *I. tenuituba* occur there in discrete patches within a matrix of sagebrush and gambel oak scrub vegetation, and hybrid individuals are rare (Aldridge, 2005). The populations at GR from which plants were drawn represent the entirety of *Ipomopsis* populations at that site. *Ipomopsis* at GR flower from late May through late June. Poverty Gulch (PG) is a tributary of the Slate River in Gunnison County, CO, and ranges between 2900 and 3250 m a.s.l. Morphological and molecular information from PG confirm that the two species lie at opposite ends of the elevational range, with an extensive hybrid swarm between them (Campbell et al., 1997; Wu and Campbell, 2005). The upper boundary of the PG contact site is defined by the altitudinal limit of *Ipomopsis* at that site; however, the lower limit was arbitrary, as *I. aggregata* occurs for many kilometers down the valley. Flowering at PG is from late June to late July, so incorporating plants from both sites into a crossing design requires keeping potted plants from GR flowering long past their usual season. Both species are protandrous, with synchronous anther dehiscence. Anther mass correlates strongly with pollen grain number in *I. aggregata* (Campbell, 1992), and the species have similar sized pollen grains (Alarcón and Campbell, 2000). There is one notable difference in floral morphology between the sites; styles of GR *I. aggregata* are significantly longer than those of GR *I. tenuituba* and both species at PG (29.9 mm for GR agg vs. 23.4–24.0 mm for all others. ANOVA: $F_{3,223} = 74.04$, $P < 0.001$).

Crosses—Our general methodology involved making hand pollinations on potted plants in a portable greenhouse that excluded natural pollinators. Crosses took two forms. Competitive crosses consisted of a 1:1 mixture of *I. aggregata* and *I. tenuituba* pollen from either GR or PG, while noncompetitive crosses included pollen from only a single species from GR. We performed competitive crosses of GR donors on GR and PG recipients to test for pollen competition between the species at GR. We also performed competitive crosses of PG donors on GR recipients as a control for a possible effect of site. Noncompetitive crosses of GR donors on GR recipients were included to test for interfertility of the species at GR and as controls for maternal plant seed set for competitive crosses. A previous study examined both competitive and noncompetitive crosses of PG donors on PG recipients and found no evidence for conspecific pollen advantage at that site (Alarcón and Campbell, 2000), so we did not repeat such crosses in our design. Because of a shortage of GR recipient flowers late in the season and difficulties getting enough *I. tenuituba* from PG, we also omitted noncompetitive crosses of PG donors on GR recipients. Because previous experiments have shown no pollen fitness differences between 6-phosphoglucose dehydrogenase (6-PGD) alleles (Alarcón and Campbell, 2000; Campbell et al., 2003), we did not test explicitly for such an effect in this study.

Candidate plants were genotyped at the slower-migrating locus of 6-PGD (Campbell and Dooley, 1992). We identified four alleles: extra-slow (XS), slow (S), medium (M), and fast (F), and attempted to collect slow and medium homozygotes. However, to obtain sufficient numbers of plants at similar phenological stage and to minimize the number of extra plants collected from GR to replace losses due to transplant shock, we used plants with any genotype other than SM. Plants were potted in the field and transported to the Rocky Mountain Biological Laboratory (110 km from GR, 25 km from PG) where they were kept in a portable greenhouse, watered and fertilized ad lib. Because we had data from a similar experiment conducted on only PG plants (Alarcón and Campbell, 2000), we emphasized gathering data from GR. Consequently, genotyping and collecting did not begin at PG until past peak flowering, especially in the *I. tenuituba* populations, and we were able to successfully pot fewer plants from PG (Table 1).

Competitive pollen mixtures were prepared using donors from either GR or PG, but no mixtures included donors from both sites. Freshly dehisced anthers were collected from 3–4 plants of each species, and weighed. The total number of anthers of each species was adjusted until the mass was equal for the two species, then all anthers were combined in a clean microcentrifuge tube and mixed thoroughly. Because pollen grain size is similar for both species, equal masses should yield approximately equal numbers of pollen grains (Alarcón and Campbell, 2000). Donors were chosen so that the paternity of each seed (i.e., *I. aggregata* or *I. tenuituba*) could be determined from its genotype, but groups of donors were not fixed. For example, a competitive mixture of GR *I. aggregata* MM homozygotes + *I. tenuituba* SS and XSS would allow paternity to be determined for all progeny because donors of each species contribute different alleles. In this example, on an MM recipient, any offspring carrying an XS or S allele would have been sired by *I. tenuituba*. Heterozygotes were always grouped in donor pools with homozygotes possessing the more common of the heterozygotes' two alleles (i.e., XSS with SS never with MM or MF). Donor groups consisted of any plants of suitable species/genotype each day.

Noncompetitive mixtures included anthers from 3–4 donors of the same genotype and species. No plant received a mixture of pollen that included its own. Pollen was applied to stigmas in saturating amounts, using a round toothpick (Campbell and Halama, 1993). Each kind of cross (i.e., GR *tenuituba* vs. GR *aggregata*), competitive as well as noncompetitive, used donors of each genotype combination (i.e., GR *tenuituba* S vs. GR *aggregata* M, and GR

TABLE 1. Numbers of *Ipomopsis* plants of various 6-phosphoglucose dehydrogenase genotypes used as recipients in pollen competition crosses. Site: GR = Grizzly Ridge, Montrose Co., CO; PG = Poverty Gulch, Gunnison Co., CO. Species: t = *I. tenuituba*; a = *I. aggregata*. Genotype: letters refer to electrophoretic alleles (XS = extra slow, S = slow, M = medium, F = fast).

Site	Species	XSS	SS	SF	MM	MF	FF
GR	t	1	5	1	3	2	2
	a		3	4	8	1	
PG	t		3		1		
	a		4		6		

tenuituba M vs. GR *aggregata* S; Table 2). This resulted in eight cross types: four competitive cross types (2 sites × 2 alleles; Table 2) and four noncompetitive cross types (2 species × 2 alleles). All fruits were collected before dehiscence, and seeds counted. Seeds from noncompetitive crosses were presumed to have been sired by the species and genotypes represented by the donors. Seeds produced from competitive crosses were genotyped at the 6-PGD locus to determine paternity.

Data analyses—Competitive crosses were performed in a design that resulted in six combinations of recipient site/recipient species/donor site ([From GR 2 recipient species × 2 donor sites] + [From PG 2 recipient species × 1 donor site]). We tested for conspecific pollen advantage by comparing the proportion of seeds sired by *I. aggregata* to the null expectation of 0.5 assuming no advantage or disadvantage. The proportion of seeds sired by *I. aggregata* donors (P-agg) was compared to 0.5 using a one-sample *t* test for each of the six combinations. Conspecific pollen advantage would result in departures above 0.5 for *I. aggregata* recipients and below 0.5 for *I. tenuituba* recipients. In this analysis, the recipient plant was the unit of replication, because individual seeds could not be assumed to be independent of other seeds from the same fruit. The total number of seeds from a given recipient, therefore, was not important to the analysis. One sample *t* tests were used to test for the departure of each type of cross from 0.5 seeds sired by *I. aggregata*.

In addition to examining conspecific pollen advantage in mixed pollinations, we also compared the seed production from different kinds of pollinations. Crosses involving GR donor pollen comprised six types: two competitive and four noncompetitive. All types of crosses were performed on all maternal recipient plants of each type (GR or PG; described later), creating a fully crossed design. The mean seed production for each donor cross type was expressed as a difference from the whole-plant mean for each GR recipient plant to control for differences in overall seed production among maternal plants. Because each cross type involved a different mix of donor pollen and the differences from plant means controlled for maternal effects on overall seed production, each cross type on each recipient plant was treated as an independent replicate. We performed two-way ANOVAs for seed production on cross type and recipient species, with two models. The first ANOVA compared the two competitive cross types to the four noncompetitive cross types; the second compared noncompetitive *I. tenuituba* pollinations to noncompetitive *I. aggregata* pollinations and excluded the competitive types. The interaction of cross type and recipient species in the latter case also tested for an effect of heterospecific vs. conspecific pollination on seed production. Poverty Gulch plants received only competitive crosses, and PG pollen participated only in competitive crosses, so PG recipients and pollen mixes were excluded from these analyses. The ANOVA was performed using the computer program R (R Development Core Team, 2004).

TABLE 2. Pollen mixtures used in pollen competition crosses performed on *Ipomopsis* plants from two sites in western Colorado, USA. Pollen was pooled from 3–4 donors and applied to recipient stigmas by hand. Donor mixes are labeled as follows: Site—Grizzly Ridge, Montrose County (GR), Poverty Gulch, Gunnison County (PG); species—*I. aggregata* (agg), *I. tenuituba* (ten); 6-phosphoglucose dehydrogenase electrophoretic allele—slow (S), medium (M). Mixes consisted of 1:1 ratios of each type. Rare alleles extra-slow (XS) and fast (F) were present in some mixes, but could not be analyzed separately. Roman numerals identify types of crosses (see Results).

Recipients	Donor mixes	*I. tenuituba* alleles	*I. aggregata* alleles
GR & PG	I. GRtenS + GRaggM	XS, S, F*	M, F*
GR & PG	II. GRtenM + GRaggS	M, F*	S, F*
GR	VII. PGtenS + PGaggM	S	M
GR	VIII. PGtenM + PGaggS	M	S

* No donor mixtures included F alleles from both species.

Altogether, 62 plants were used as pollen donors and 44 as recipients for all competitive crosses. The number of seeds obtained from crosses involving any particular donor varied from 16–815, potentially biasing the results if certain donors had more fit pollen and thus were associated with high rates of seed production. Therefore, we tested for an effect of total seed production on P-agg in competitive crosses. Because pollen from multiple donors of each species was pooled, it was impossible to determine exact paternity, but we were able to calculate the total number of seeds produced, and the mean of P-agg, for all crosses involving each donor plant. P-agg was arcsine square-root transformed (Sokal and Rohlf, 1995), then plotted in box plots of conspecific donors within sites (i.e., all GR *aggregata* donors) and inspected for outliers. An outlier would have indicated a donor that consistently participated in crosses that resulted in an extremely high or low proportion of offspring sired by *I. aggregata*. We also tested for an effect of seed production on P-agg with linear, exponential, and logarithmic regressions.

Competitive crosses yielded a total of 2288 seeds. There was no evidence that any donor consistently participated in competitive crosses that produced an unusually high proportion of *I. aggregata* offspring (P-agg) when compared to conspecific donors from the same site or that total seeds produced affected the proportion of *I. aggregata* offspring. Box plots of P-agg grouped by donor species within site had no outliers, and no regression (linear, exponential, logarithmic) of P-agg over seed production had a coefficient significantly different from zero.

RESULTS

Instead of a conspecific pollen advantage, there was evidence of an asymmetrical seed-siring advantage for *I. aggregata* from GR. *Ipomopsis aggregata* donors from GR sired 70–80% of seeds in competitive crosses, irrespective of recipient site or species (Fig. 1). This was contrary to both the null expectation of 50% and the alternative hypothesis of reduced *I. aggregata* pollen success on *I. tenuituba* recipients. Seed-siring proportions were significantly higher than 0.5 for *I. aggregata* donors from GR, but not from PG (Fig. 1), indicating that the difference was not due to characteristics of the GR recipients alone.

Noncompetitive (= pure) crosses resulted in higher average seed production than did competitive (= mixed) crosses of GR donors (Table 3, mixed vs. pure crosses; Fig. 2). There was no effect of recipient species (Table 3, main effect of Recip. Species), and the effect of mixed vs. pure cross type was consistent across recipient species (Table 3, interaction). Seed production was similar among noncompetitive cross types (Table 3, pure *I. tenuituba* vs. *I. aggregata* crosses, main effect of Cross), regardless of whether the donor was conspecific or heterospecific to the recipient (Table 3, interaction; Fig. 2), indicating that the species are fully interfertile at GR. Noncompetitive crosses overall averaged 4.3 more seeds per fruit than all competitive crosses (Table 2), even those including PG donors, a 75% increase (9.9 vs. 5.6). This effect was due to crosses with GR donors only (9.9 vs. 4.6, cross types I and II; Table 2), as competitive crosses of PG donors on GR recipients (cross types VII and VIII; Table 2) set very near the overall mean (7.3 all crosses vs. 6.8 PG competitive).

DISCUSSION

We observed markedly different patterns of pollen success and fitness of mixed pollen loads at Grizzly Ridge and Poverty

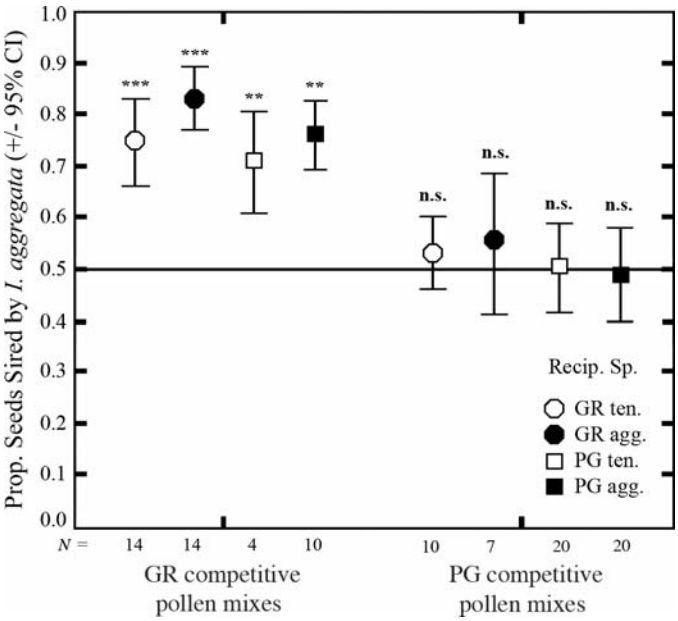

Fig. 1. Proportion of seeds sired by *Ipomopsis aggregata* pollen donors (P-agg) in mixed-species pollinations of recipient plants from two sites in the western Rocky Mountains, USA. All pollen mixtures contained equal amounts of pollen from two species (agg = *Ipomopsis aggregata*; ten = *I. tenuituba*). N = number of recipient plants; Donor pollen source indicates the site from which donor plants were collected (GR = Grizzly Ridge, Montrose Co., CO; PG = Poverty Gulch, Gunnison Co., CO). Symbols indicate P value of 1-sample t tests of the null expectation of P-agg = 0.5 (***: uncorrected $P < 0.001$; **: $P < 0.01$; ns = $P > 0.05$). All cases marked ** or *** were also significant using a sequential Bonferroni correction. Paternity of seeds was determined by genotyping at a 6-phosphoglucose dehydrogenase locus using enzyme electrophoresis. Recipients producing fewer than five seeds were excluded. Data for PG recipients of PG pollen taken from Alarcón and Campbell (2000).

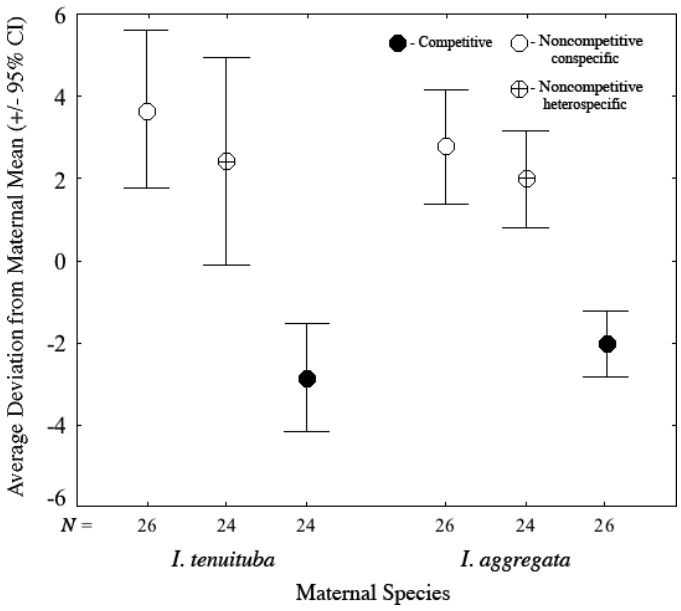

Fig. 2. Seed production measured as departure from the average seeds per fruit for maternal plants receiving competitive (mixed-species) and noncompetitive (single-species) pollinations of *Ipomopsis* plants at Grizzly Ridge, Montrose Co., CO. Departure from mean is within a recipient plant; bars are 95% CI for mean of all recipient plants' departures. An average departure of zero corresponds to 7.3 seeds per fruit for that cross type. N = number of crosses on all recipients (2 types of competitive, 4 types of noncompetitive); open circles = noncompetitive pollen loads with conspecific donors and recipients; hatched circles = noncompetitive pollen loads with heterospecific donors and recipients; closed circles = competitive (1:1) pollen loads.

TABLE 3. Two-way ANOVAs comparing the residual (difference from overall plant mean) seed set of mixed- and pure-species pollen loads on *Ipomopsis aggregata* and *I. tenuituba* from western Colorado, USA. Two types of each pollination were performed because pollen donors were separated into groups according to their electrophoretic alleles at the 6-phosphoglucose dehydrogenase locus. The top ANOVA compared mixed loads (2 types) to pure loads (4 types: 2 alleles × 2 species); the bottom ANOVA compared *I. tenuituba* (*ten*) pure pollen loads (2 types) to *I. aggregata* (*agg*) pure pollen loads (2 types).

Source	df	Type III SS	MS	*F*	*P*
Mixed vs. pure crosses					
Recip. Species	1	1.20	1.20	0.079	0.778
Cross	1	573.77	573.77	37.935	<0.001***
Recip. Species × Cross	1	19.56	19.56	1.293	0.258
Error	146	2208.28	15.13		
Pure *ten* vs. *agg* crosses					
Recip. Species	1	3.00	3.00	0.142	0.707
Cross	1	6.99	6.99	0.329	0.567
Recip. Species × Cross	1	16.92	16.92	0.797	0.373
Error	146	3098.47	21.22		

Gulch. Our results for PG donors are consistent with previous work at that site (Alarcón and Campbell, 2000), in which species sired seed in direct proportion to their fraction in mixed pollen loads, and there was no reduction in seed set from mixed pollen loads. In contrast, *I. aggregata* donors from GR sired approximately 80% of seeds on conspecific recipients and 70% of seeds on *I. tenuituba* recipients, and mixed pollen loads of GR donors set less than half as many seeds as noncompetitive loads. This effect was present only in competitive pollen mixtures. Single-species pollen loads sired similar numbers of seeds regardless of donor or recipient species, indicating that the species are fully interfertile at both GR and PG. The differential pollen success in competitive mixtures should promote asymmetrical gene flow at GR from *I. aggregata* into *I. tenuituba*, while the fitness reduction of mixed pollinations should depress hybrid formation over all. The quantitative effect will depend on how often mixed pollen loads are deposited and on the performance of hybrid pollen (Campbell et al., 2003).

Asymmetric barriers to gene flow in plants appear to be common (Tiffin et al., 2001), but we know of only one other study reporting variation in endogenous reproductive isolating mechanisms between contact sites of the same species, in this case among oaks. Williams et al. (2001) found strong asymmetries in pollen performance only in a unimodal contact site between *Quercus grisea* and *Q. gambelii* that contributed to more hybridization at that site relative to a bimodal site of the same two species. In contrast, we found asymmetrical pollen success at a site where intermediate individuals are

scarce, and no differences at one where they are abundant. Whether the combination of unilateral pollen success and reduced fitness of mixed pollen loads we observed constitutes a barrier sufficient to explain the difference in hybridization rate between Grizzly Ridge and Poverty Gulch is unclear. Modeling the dynamics of a contact site characterized by the con- and heterospecific pollen success rates and relative fitness of pure and mixed pollen loads we measured in this study would require knowing how often mixed pollen loads are deposited and the fitness of hybrid pollen. There is evidence that even at PG, where no conspecific pollen advantage was found for either species, and no fitness reduction for mixed pollen loads (this study; Alarcón and Campbell, 2000), F_1 and F_2 pollen sires proportionally fewer seeds than parental species in mixed pollinations (Campbell et al., 2003). Hybrid breakdown through reduced pollen fitness might also restrict gene flow at GR, though we have no data as yet on the fitness of hybrid pollen there, as natural hybrids are unknown at that site.

We can estimate the effects of the asymmetries we measured on the formation of hybrids in the first generation of mating (F_1) between two equal-sized pure populations of the parent species, although we lack data on the fitness of hybrid pollen on parental stigmas that would allow us to extend our estimates beyond the F_1 generation. We constructed a simple analytical model that assumed the following: pollen was equally likely to travel to flowers of the two species (and hence equally likely to travel to conspecific and heterospecific flowers), all mixed pollen loads were 1:1, and there were no hybrids already present. We incorporated the observed fitness of these mixed pollen loads and the relative competitive ability of each species' pollen at each site. The formation of hybrids in this model is determined by the proportion of pollinations that are mixed (P). The equations are as follows:

$$F_{1\ \text{conspecific}} = 0.5[PA_{con}M + (1-P)0.5] + 0.5[PT_{con}M + (1-P)0.5]$$

$$F_{1\ \text{hybrid}} = 0.5[P(1-A_{con})M + (1-P)0.5] + 0.5[P(1-T_{con})M + (1-P)0.5],$$

where F_1 = the first offspring generation, A_{con} = the proportion of offspring sired by *I. aggregata* in mixed pollen loads on *I. aggregata*, T_{con} = the proportion of offspring sired by *I. tenuituba* in mixed pollen loads on *I. tenuituba*, and M = the relative fitness of mixed pollen loads. Consider the first equation, for $F_{1\ \text{conspecific}}$. The first half of the equation represents the half of pollen loads that will be deposited on *I. aggregata*. In that case, mixed pollen loads occur at frequency P, and *I. aggregata* pollen will sire conspecific offspring at a rate of A_{con}, which is then discounted by M, the relative fitness of mixed pollen loads. Pure pollen loads occur at frequency $1 - P$, and 0.5 of those will lead to production of conspecific offspring regardless of the recipient species. The second half of the equation represents the half of the pollen loads that will be deposited on *I. tenuituba* stigmas, in which case *I. tenuituba* pollen in mixed loads will sire conspecific offspring at rate T_{con}. The sum of these two numbers represents the proportion of the F_1 generation that is pure parental species. For equation $F_{1\ \text{hybrid}}$, the process is identical, except that heterospecific pollen sires hybrid seeds at rate $1 - A_{\text{Con}}$ or $1 - T_{con}$, in mixed loads on *I. aggregata* or *I. tenuituba*, respectively. The relative size of the F_1 generation is simply the sum of $F_{1\ \text{conspecific}}$ and $F_{1\ \text{hybrid}}$, and the percentage of hybrids is the proportion of the F_1 generation made up by the $F_{1\ \text{hybrid}}$.

In real populations, P would depend on the frequency of interspecific flights by pollinators and the degree of pollen carryover (Campbell et al., 2002). While this model is too simple to capture realistic variation in pollen mixtures, it can suggest the range of outcomes that might be possible for different values of P. We varied P from 0 to 1 for both sites. At PG, a site with no differences in seed-siring success between species and no reduced seed set from mixed pollinations ($A_{\text{Con}} = T_{\text{Con}} = 0.5$; $M = 1$), hybrids make up 50% of the F_1 generation for all values of P from 0 to 1. At GR, $P = 0$ results in an F_1 generation identical to that at PG, because there are no mixed pollinations and hence no fitness consequence and no asymmetrical seed-siring success. Increasing P from 0 to 1 at GR ($A_{\text{Con}} = 0.8$; $T_{\text{Con}} = 0.3$; $M = 0.5$) results in a 50% reduction of the overall size of the F_1 generation, but a decrease of only 5% in the proportion of hybrids (45% vs. 50%). The proportion of these hybrids that have *I. tenuituba* as the seed parent increases from 50% at $P = 0$ to 77.8% at $P = 1$ due to the seed siring advantage of *I. aggregata* pollen in mixed pollen loads. These results suggest that the differences in postmating reproductive isolation between GR and PG are probably not sufficient to explain by themselves the extreme differences in the frequency of hybrids at the two sites (Aldridge, 2005).

The unilateral seed siring advantage of *I. aggregata* in mixed pollen loads represents an asymmetrical barrier to gene flow at GR that is not present at PG. Mixed pollen deposition on *I. aggregata* stigmas would result in very few hybrid offspring, while hybrids would be disproportionately common when mixed pollen loads were deposited on *I. tenuituba* stigmas. This would result in a greater likelihood of *I. aggregata* nuclear genes introgressing onto the *I. tenuituba* cytoplasmic background than vice versa, and many fewer pure *I. tenuituba* offspring. This situation might be expected to result in a greater likelihood of genetic swamping of *I. tenuituba* by *I. aggregata* at GR than at PG. Data on the competitive performance of hybrid pollen from GR would further clarify this matter. Hybrid pollen at PG has a competitive disadvantage in mixed pollinations (Campbell et al., 2003). These species are closely related and show very little genetic divergence within contact sites (Wolf et al., 1997; Wu and Campbell, 2005; G. Aldridge and D. Campbell, unpublished data), so positive identification of F_1 and backcross individuals is difficult. It might be that the difference in pollen competition between sites is the result of greater introgression at PG than at GR and therefore is a byproduct and not a cause of different degrees of reproductive isolation at the two sites. If this is the case, the same asymmetry in pollen fitness must have once existed at PG and broken down due to gene flow. The continued presence of the asymmetry at GR then would indicate less gene flow at that site, due either to stronger pre-mating isolation or shorter period of contact between the species at that site. Studies of pollinator behavior indicate very strong pre-mating isolation at GR compared to PG (Campbell and Aldridge, 2006). The reduced success of F_1 pollen found by Campbell et al. (2003) suggests that the parental species at PG have divergent alleles for pollen fitness that, when in heterozygous condition in an F_1 hybrid, produce inferior pollen performance relative to either parent. Once again, data on the competitive performance of pollen from artificially produced F_1 hybrids from GR might

shed light on the temporal development of *Ipomopsis* hybrid zones.

Other studies have reported asymmetrical seed siring success in mixed pollinations and have attributed it to ecological stress (Williams et al., 2001), polyploidy (Husband et al., 2002), or style length differences (Wolf et al., 2001) causing pollen of one species to perform poorly on stigmas of the other. All these studies reported a lack of reduction in seed set from mixed species pollinations and so concluded that differential seed siring success was not caused by selective abortion of zygotes (Hauser et al., 1997) or ovule usurpation (Waser and Price, 1991), but rather by higher growth or survival rates by one species' pollen tubes (Wang and Cruzan, 1998). We did observe a marked reduction in seed set from mixed pollinations of GR donors; however, the lack of an effect of recipient species (Fig. 2) argues against zygote abortion as the cause. For that to be true, maternal plants of both species would have to prejudicially abort zygotes sired by *I. tenuituba* from GR, but not from PG. Higher seed siring success in long- vs. short-styled species is common (Williams and Rouse, 1988; Sorensson and Brewbaker, 1994; Wolf et al., 2001) and might account for the observed unilateral advantage of GR *I. aggregata* pollen, since that type of plant has longer styles than the others. However, that still does not explain the reduction in overall seed set from mixed pollinations. Competing *I. aggregata* donors from GR and PG would further test the style length hypothesis, because PG *I. aggregata* styles are similar in length to those of GR *I. tenuituba* and so should produce the same pattern of unilateral advantage if it is due to style length. If possible, direct measurements of ovule abortion would help to determine if the difference in seed siring success we observed is due to pre- or postzygotic mechanisms.

The question remains as to whether the pollen competition differences we observed reflect differences in the breakdown or the buildup of reproductive isolation. Wolf et al. (1997) surveyed *Ipomopsis aggregata–I. tenuituba* zones of sympatry throughout the western USA using RFLP markers and found no evidence that conclusively indicated whether they were zones of secondary or primary contact. We (unpublished data) have examined PG and GR in detail using RAPD markers and found no markers diagnostic for either species or either site. In either case, it appears that there is local variation in reproductive isolating mechanisms that can affect gene flow.

In summary, we found that pollen competition dynamics can vary among contact sites within a species pair. While the difference was not large, it was in a direction consistent with the difference in rate of hybrid formation, lending support to the suggestion (Arnold, 1997) that pollen interactions play a role in determining reproductive isolation. The possibility that reproductive isolation varies among contact sites of the same species pair should encourage conservation biologists and agricultural managers to consider a broader range of ecological circumstances when assessing the impacts of species introductions or control of engineered genes.

LITERATURE CITED

ALARCÓN, R., AND D. R. CAMPBELL. 2000. Absence of conspecific pollen advantage in the dynamics of an *Ipomopsis* (Polemoniaceae) hybrid zone. *American Journal of Botany* 87: 819–824.

ALDRIDGE, G. 2005. Variation in frequency of hybrids and spatial structure among *Ipomopsis* (Polemoniaceae) contact sites. *New Phytologist* 167: 279–288.

ARNOLD, M. L. 1992. Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23: 237–261.

ARNOLD, M. L. 1997. Natural hybridization and evolution. Oxford University Press, New York, New York, USA.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113–148.

BERNASCONI, G. 2004. Seed paternity in flowering plants: an evolutionary perspective. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 149–158.

CAMPBELL, D. R. 1992. Variation in sex allocation and floral morphology in *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany* 79: 516–521.

CAMPBELL, D. R., R. ALARCÓN, AND C. A. WU. 2003. Reproductive isolation and hybrid pollen disadvantage in *Ipomopsis*. *Journal of Evolutionary Biology* 16: 536–540.

CAMPBELL, D. R., AND G. ALDRIDGE. 2006. Floral biology in hybrid zones. *In* L. Harder and S. C. H. Barrett [eds.], The ecology and evolution of flowers, in press. Oxford University Press, Oxford, UK.

CAMPBELL, D. R., AND J. L. DOOLEY. 1992. The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. *American Naturalist* 139: 735–748.

CAMPBELL, D. R., AND K. J. HALAMA. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74: 1043–1051.

CAMPBELL, D. R., N. M. WASER, AND E. J. MELÉNDEZ-ACKERMAN. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *American Naturalist* 149: 295–315.

CARNEY, S. E., S. A. HODGES, AND M. L. ARNOLD. 1996. Effects of differential pollen tube growth on hybridization in the Louisiana irises. *Evolution* 50: 1871–1878.

COYNE, J. A., AND H. A. ORR. 2004. Speciation. Sinauer, Sunderland, Massachusetts, USA.

CRUZAN, M. B., AND S. C. H. BARRETT. 1996. Postpollination mechanisms influencing mating patterns and fecundity: an example from *Eichhornia paniculata*. *American Naturalist* 147: 576–598.

ELLSTRAND, N. C., H. C. PRENTICE, AND J. F. HANROCK. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539–563.

EMMS, S. K., S. A. HODGES, AND M. L. ARNOLD. 1996. Pollen-tube competition, siring success, and consistent asymmetric hybridization in Louisiana irises. *Evolution* 50: 2201–2206.

GRANT, V., AND K. GRANT. 1965. Flower pollination in the phlox family. Columbia University Press, New York, New York, USA.

GRANT, V., AND D. H. WILKEN. 1988. Natural hybridization between *Ipomopsis aggregata* and *Ipomopsis tenuituba* (Polemoniaceae). *Botanical Gazette* 149: 213–221.

HARRISON, R. G., AND S. M. BOGDANOWICZ. 1997. Patterns of variation and linkage disequilibrium in a Field Cricket hybrid zone. *Evolution* 51: 493–505.

HAUSER, T. P., R. B. JORGENSEN, AND H. ØSTERGÅRD. 1997. Preferential exclusion of hybrids in mixed pollinations between oilseed rape (*Brassica napus*) and weedy *B. campestris* (Brassicaceae). *American Journal of Botany* 84: 756–762.

HEWITT, G. M. 1988. Hybrid zones—natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3: 158–167.

HUSBAND, B. C., D. W. SCHEMSKE, T. L. BURTON, AND C. GOODWILLIE. 2002. Pollen competition as a unilateral reproductive barrier between sympatric diploid and tetraploid *Chamerion angustifolium*. *Proceedings of the Royal Society of London, B, Biological Sciences* 269: 2565–2571.

JIGGINS, C. D., AND J. MALLETT. 2000. Bimodal hybrid zones and speciation. *Trends in Ecology and Evolution* 15: 250–255.

LEVIN, D. A., J. FRANCISCO-ORTEGA, AND R. K. JANSEN. 1996. Hybridization and the extinction of rare plant species. *Conservation Biology* 10: 10–16.

MARSHALL, J. L., M. L. ARNOLD, AND D. J. HOWARD. 2002. Reinforcement: the road not taken. *Trends in Ecology and Evolution* 17: 558–563.

MOONEY, H. A., AND E. E. CLELAND. 2001. The evolutionary impact of

invasive species. *Proceedings of the National Academy of Sciences, USA* 98: 5446–5451.

R DEVELOPMENT CORE TEAM. 2004. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org [accessed 2 June 2005].

RAMSEY, J., H. D. BRADSHAW, AND D. W. SCHEMSKE. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* 57: 1520–1534.

RIESEBERG, L. H., AND S. C. CARNEY. 1998. Tansley review 102: plant hybridization. *New Phytologist* 140: 599–624.

SONG, Z., B. LU, Y. ZHU, AND J. CHEN. 2002. Pollen competition between cultivated and wild rice species (*Oryza sativa* and *O. rufipogon*). *New Phytologist* 153: 289–296.

SORENSSON, C. T., AND J. L. BREWBAKER. 1994. Interspecific compatibility among 15 *Leucaena* species (Leguminosae: Mimosoideae) via artificial hybridizations. *American Journal of Botany* 81: 240–247.

TIFFIN, P., M. S. OLSON, AND L. C. MOYLE. 2001. Asymmetical crossing barriers in angiosperms. *Proceedings of the Royal Society of London, B, Biological Sciences* 268: 861–867.

WANG, J., AND M. B. CRUZAN. 1998. Interspecific mating in the *Piriqueta caroliniana* (Turneraceae) complex: effects of pollen load size and composition. *American Journal of Botany* 85: 1172–1179.

WASER, N. M., AND M. V. PRICE. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *American Journal of Botany* 78: 1036–1043.

WATANO, Y., A. KANAI, AND N. TANI. 2004. Genetic structure of hybrid zones between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae) revealed by molecular hybrid index analysis. *American Journal of Botany* 91: 65–72.

WILLIAMS, J. H., W. J. BOECKLEN, AND D. J. HOWARD. 2001. Reproductive processes in two oak (*Quercus*) contact zones with different levels of hybridization. *Heredity* 87: 680–690.

WILLIAMS, J. H., W. E. FRIEDMAN, AND M. L. ARNOLD. 1999. Developmental selection within the angiosperm style: using gamete DNA to visualize interspecific pollen competition. *Proceedings of the National Academy of Sciences, USA* 96: 9201–9206.

WILLIAMS, E. G., AND J. L. ROUSE. 1988. Disparate style lengths contribute to isolation of species in *Rhododendron*. *Australian Journal of Botany* 36: 183–192.

WOLF, P. G., D. R. CAMPBELL, N. M. WASER, S. D. SIPES, T. R. TOLER, AND J. K. ARCHIBALD. 2001. Tests of pre- and postpollination barriers to hybridization between sympatric species of *Ipomopsis* (Polemoniaceae). *American Journal of Botany* 88: 213–219.

WOLF, P. G., R. A. MURRAY, AND S. D. SIPES. 1997. Species-independent, geographical structuring of chloroplast DNA haplotypes in a montane herb *Ipomopsis* (Polemoniaceae). *Molecular Ecology* 6: 283–291.

WOLF, P. G., P. S. SOLTIS, AND D. E. SOLTIS. 1993. Phylogenetic significance of chloroplast restriction site variation in the *Ipomopsis aggregata* complex and related species (Polemoniaceae). *Systematic Botany* 18: 652–662.

WU, C. A., AND D. R. CAMPBELL. 2005. Cytoplasmic and nuclear markers reveal contrasting patterns of spatial and genetic structure in an *Ipomopsis* hybrid zone. *Molecular Ecology* 14: 781–792.