Predicting Patterns of Mating and Potential Hybridization from Pollinator Behavior

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ABSTRACT: Hybridization in flowering plants is determined in part by the rate at which animal pollinators move between species and by the effectiveness of such movements in transferring pollen. Pollinator behavior can also influence hybrid fitness by determining receipt and export of pollen. We incorporated information on pollinator effectiveness and visitation behavior into a simulation model that predicts pollen transfer between *Ipomopsis aggregata*, *Ipomopsis tenuituba*, and hybrids. These predictions were compared with estimates of pollen transfer derived from movement of fluorescent dyes in experimental plant arrays. Interspecific pollen transfer was relatively uncommon in these arrays, whereas transfer between hybrids and the parental species was at least as common as conspecific transfer. Backcrossing was asymmetrical; *I. aggregata* flowers frequently received mixed loads of hybrid and conspecific pollen. The simulation suggests that these patterns of pollen transfer are largely explained by the visitation sequences of hummingbird and insect pollinators, with little contribution from mechanical isolation. Pollen receipt by hybrids exceeded that of both parental species in a year when pollinators preferred to visit F_1 and F_2 hybrids and was intermediate in another year when they preferred to visit *I. aggregata*. This suggests that natural variation in pollination may produce spatiotemporal variation in hybridization and hybrid fitness.

Keywords: ethological isolation, hummingbird, hybridization, *Ipomopsis*, pollen carryover, pollen transfer.

The rate of hybridization between related species depends on the strength of prezygotic and postzygotic reproductive barriers. In flowering plants, the initial stages of prezygotic isolation are often tied to the behavior of animal pollinators. Production of hybrids requires first that pollen be transferred to stigmas of another species, which in turn depends on how frequently pollinators fly between flowers of these species and on the effectiveness of pollen transfer between them. As a result, hybridization is opposed by any tendency of pollinators to move only between conspecific flowers ("ethological isolation" sensu Grant 1949) and by any difference in floral morphology, such as in the placement of reproductive parts, that reduces pollen transfer to conspecific flowers ("mechanical isolation"). If pollen is transferred between species, formation of hybrid zygotes can still be prevented by postpollination processes. Hybrids will not be formed if heterospecific pollen is incapable of germinating on the stigma or producing pollen tubes that grow down the style. Even if heterospecific pollen fertilizes when present by itself, it may not do so when conspecific pollen is mixed with it on the stigma. Such a mechanism was hypothesized by Arnold (1997) to play a major role in limiting hybridization of plants.

These pollination and postpollination elements of prezygotic barriers have long been understood (Stebbins 1950) and examples identified (reviewed in Levin 2000), but the relative importance of different elements has seldom been quantified in a natural setting. In large part, this reflects our ignorance of patterns of pollen transfer in natural hybrid zones. For example, a 1995 review of hybrid fitness by Arnold and Hodges uncovered no studies of pollination success. Indeed, we have few data from natural populations on mating patterns and their influence on hybridization, whether for plants or for animals (Mallet et al. 1998). Several recent studies have investigated postpollination reproductive isolation in plants. They use experimental pollinations with mixed loads of conspecific and heterospecific pollen, followed by genotyping of progeny to determine paternity (e.g., Carney et al. 1994; Rieseberg et al. 1995; Hauser et al. 1997). But the interpretation of such studies depends on how often mixed loads of pollen are deposited in the first place. We are aware of no such data for naturally hybridizing species in which pollen competition has been studied (Arnold 1997). A few esti-

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mates do exist of hybridization rates from molecular markers (Nason et al. 1992; Rieseberg et al. 1998; Carney et al. 2000), but it is difficult to discern the roles of pollination versus postpollination isolating mechanisms because of the absence of information on pollinator behavior and pollen transfer. Differences in floral preferences of different pollinators can suggest the potential for ethological isolation (e.g., Fulton and Hodges 1999; Schemske and Bradshaw 1999), but quantifying the strength of reproductive isolation also requires information on interspecific flights and rates of pollen transfer. Only a few studies have provided the requisite data on interspecific flights (e.g., Leebens-Mack and Milligan 1998; Wesselingh and Arnold 2000), and even in these cases we do not know the resulting effects on pollen transfer.

This article is part of a study comparing pollination and postpollination forms of reproductive isolation by using two naturally hybridizing species of *Ipomopsis* (Polemoniaceae) as a model system. Like others, we have examined the success of heterospecific pollen when present in pure and mixed stigmatic loads (Alarcón and Campbell 2000). Here we focus on the pollination component to describe patterns of mating between the species. Our previous studies have described the rates at which pollinators fly between flowers of the two species (Campbell et al. 1997, 1998; Meléndez-Ackerman et al. 1997; Meléndez-Ackerman and Campbell 1998) and the effectiveness of pollinators at transferring pollen during these interspecific flights (Campbell et al. 1998). We now use this information to build a simulation model that predicts patterns of pollen transfer between the two parental species and their hybrids. We compare these predictions with estimates of pollen transfer in experimental arrays containing the parental species and hybrids. We address three questions. First, how does the rate of interspecific pollen transfer compare with that of conspecific pollen transfer, and how much does it depend on ethological isolation versus mechanical isolation? Second, is the potential for backcrossing symmetrical or asymmetrical? Finally, how frequently do stigmas receive mixed loads of pollen that would generate potential for pollen competition? In addition to addressing these questions, we compare hybrids and parental species in amounts of pollen received and exported. These measures of pollination success are one component of plant fitness and thus help to inform us about the relative fitness of hybrids (Arnold and Hodges 1995).

Methods

Study System

Ipomopsis aggregata and *Ipomopsis tenuituba* form natural hybrid swarms in several contact sites scattered throughout the western United States (Grant and Wilken 1988). Plants for this study came from Poverty Gulch in Gunnison County, Colorado. In this area, *I. aggregata* ssp. *aggregata* grows in the valley floor up to 2,900 m elevation, *I. tenuituba* ssp. *tenuituba* grows from 3,100 m to 3,250 m, and populations of hybrids are found in between (Grant and Wilken 1988). All *I. aggregata* plants were collected from population L in the valley, where there are no signs of morphological introgression (Campbell et al. 1997). All *I. tenuituba* plants came from the highest-elevation populations A–C, which also show no signs of morphological introgression (Campbell et al. 1997). The flowers of *I. aggregata* have red, trumpet-shaped corolla tubes that are relatively broad and short, with anthers presented near the orifice of the tube and the stigma ranging from slightly inserted to exserted. The flowers of *I. tenuituba* have pale pink or white corollas that are narrower and longer, with anthers and stigmas inserted deep within the tube. Flowers of both species are protandrous and self-incompatible. At Poverty Gulch, the main present-day pollinators are broad-tailed hummingbirds (*Selasphorus platycercus*) and rufous hummingbirds (*Selasphorus rufus*). Although *I. tenuituba* has traits frequently associated with hawkmoth pollination (Grant 1992), hawkmoths are rare at this site; in spite of extensive field observations every year since 1991, we have witnessed moth visits to *Ipomopsis* flowers only in 1993 and 2001 (Campbell et al. 1997; Campbell and Waser 2001). When present, hawkmoths (*Hyles lineata*) are often diurnal in this high-elevation region.

Experimental Arrays

To determine patterns of pollen transfer, we set out an experimental array of potted plants for two periods, July 19–31, 1999, and July 5–19, 2000. We used fluorescent dyes (series R-103-G, Radiant Color, Richmond, Calif.) to estimate patterns of pollen transfer among flowers. Previous studies have established that these dyes serve as an excellent analog for pollen transfer in this system (Waser and Price 1982; Waser 1988). In 2000, we also characterized patterns of pollinator visitation behavior to plants in the array. These visitation sequences were incorporated into our simulation model to evaluate the extent to which interspecific pollen transfer was reduced simply by the tendency of pollinators to fly to conspecifics, that is, by ethological isolation.

Each array mimicked a situation in which the two species and hybrids are intermixed and are equally available to pollinators. Thus our study provides upper bounds on the pollination component of hybridization. The 1999 array consisted of 12 flowering plants each of *I. aggregata*, *I. tenuituba*, and natural hybrids potted from population 1 in the center of the Poverty Gulch hybrid zone, where

a wide range of floral morphologies is found (Campbell et al. 1997). The 2000 array was identical except that we used known F_1 and F_2 plants (six of each) instead of natural hybrids. These hybrid plants were produced by crosses in the greenhouse initiated in 1994 at the Rocky Mountain Biological Laboratory (RMBL). Four of the six F_1 hybrids had *I. aggregata* as the maternal parent. All plants in each array received regular watering and were fertilized once before placement in the field.

The 1999 and 2000 arrays were set up at the same site in a large meadow 1 km south of the RMBL. All pollinators we have witnessed at Poverty Gulch also occur at this site. Moreover, hummingbirds make similar choices between the two species of *Ipomopsis* at RMBL and at Poverty Gulch (Campbell et al. 1997). Plants were arranged in a square 6 × 6 grid with a spacing of 0.5 m, with individuals assigned at random to the 36 grid locations.

In each year, observations were made in three cycles, each with a different assignment of three fluorescent dye colors to the three types of plants (*I. aggregata*, hybrid, and *I. tenuituba*). These colors were rotated between the cycles in a Latin-square design. A cycle lasted for 3 d. We assigned half of the 12 plants of each type to serve as dye donors for the duration of the experiment, with the other half serving as dye recipients. These assignments were made at random, subject to the constraint that there be three F_1 and three F_2 dye donors in 2000. On the afternoon of the first day, dye was applied with a toothpick to the dehiscing anthers of all staminate phase flowers on the dye donors. Dye was again applied to newly opened staminate-phase flowers on the dye donors during the afternoon of the second day. On the mornings of the second and third days, we sampled pistillate-phase flowers from the dye recipients and brought them to the RMBL, where we scored dye loads on the stigmas under 50 × magnification (Waser and Price 1982; Campbell 1989). On the second day, we sampled 25% of all pistillate flowers on recipients, and on the third day, we sampled an additional 50%. In order to determine number and sex ratios of available flowers, we also counted staminate and pistillate flowers on each plant whenever we applied dye or sampled recipients. The plants were returned to the greenhouse for 3 d before we began the next cycle. This allowed flowers with the old dye color to abscise and fresh flowers to open for use in the next cycle of dye application.

For each recipient plant, we summed over all three cycles the total dye received from each type of donor: *I. aggregata*, hybrid, or *I. tenuituba*. Dividing the sums by the number of pistillate flowers sampled yielded mean dye receipt per flower as a function of type of donor. We analyzed the data separately for each year of the study by performing a two-way ANOVA on mean dye receipt, with the factors of type of recipient (three levels), type of donor (three levels), and the interaction. The combined data from both years were also analyzed with three-way factorial ANOVA with the additional factor of year so we could examine differences across years. We evaluated specific hypotheses with a priori contrasts on this combined data set. For each of the pure species donors, we contrasted transfer to conspecific flowers versus flowers of the alternate species to evaluate the potential for reproductive isolation. An orthogonal contrast compared the average level of backcrossing between hybrids and *I. aggregata* with the average level of backcrossing involving *I. tenuituba*. In 2000, we also performed a two-way ANOVA that separated recipients into four types (*I. aggregata*, *I. tenuituba*, F_1, and F_2). An a priori contrast was used to test the hypothesis that type of hybrid (F_1 vs. F_2) influences the level of dye receipt. All ANOVAs employed PROC GLM in SAS. To evaluate the frequency of stigmas with mixed pollen loads, we also categorized stigmas according to the type(s) of dye received.

Simultaneous information on visitation of pollinators was obtained in 2000. We observed the array for a total of 23.25 h broken into 12 periods between 0530 and 2030 hours. We witnessed 14 foraging bouts by hummingbirds (broad-tailed and rufous) and four bouts by solitary bees (*Andrena* spp.). For each animal that visited one or more flowers in the array, we recorded the identity of the visitor, the sequence of plants visited, and the number of flowers visited on each plant in the sequence. From these data, we determined how often hummingbirds and bees flew between plants of the different taxa.

We also calculated the mean visits per open flower per hour for each plant in the 2000 array. These visitation rates were compared across the four types of plant with one-way ANOVA. We then used multiple regression to examine the relationship between hummingbird visitation rate and floral traits shown to influence visitation in previous studies of this genus (Mitchell 1993; Campbell 1996; Campbell et al. 1997; Meléndez-Ackerman and Campbell 1998). Traits measured were corolla length, corolla width, 48-h nectar production (NPR), and optical density (an index of flower color). Methods are described in Mitchell (1993) and Campbell et al. (1997). We averaged values for corolla length, width, and NPR across multiple flowers (up to five) on a plant and used the averages as the independent variables in analysis.

Overview of the Simulation Model

The simulation model combined information on visitation

Table 1: Parameter values used in the simulation model

	Ipomopsis aggregata	Hybrid	*Ipomopsis tenuituba*
Probability of visiting a flower:			
1993	.39	.39	.39
2000	.27	.27	.27
Number of flowers displayed:			
1999	11	10	7
2000	10	17	11
Proportion pistillate:			
1999	.46	.45	.26
2000	.44	.37	.23
Pollen pickup:			
All	1,713	898	482

havior, we used the model to assess the extent to which interspecific transfer is limited by ethological versus mechanical isolation and to evaluate the sensitivity of pollen transfer to year-to-year variation in pollinator behavior.

In the simulation, pollinators moved through an array of plants containing equal numbers of *I. aggregata*, *I. tenuituba*, and hybrids in the same positions as in the field array, and they visited the three types of plants according to sequences we had observed in the field. Once pollinators were at a plant, their visitation behavior and pollen deposition followed rules that are extensions of the single-species model presented in Campbell (1998). Each of the three types of plants had a fixed number of flowers, with each flower on a plant assigned pistillate or staminate phase according to a probability estimated for that plant type. Once having arrived at a plant, a pollinator visited each flower with a constant probability. It picked up pollen grains from staminate phase flowers and deposited pollen regardless of sexual phase (Campbell et al. 1994) according to an exponential carryover model (see Campbell 1998 for justification). These rules and the parameter values used in implementing them are based on field observations as described in "Estimates of Parameter Values." The simulation was written in Turbo Pascal.

We ran several versions of the simulation. First, to determine whether the model accurately predicts pollen transfer in the 2000 array we established an array of 36 plants modeled after our field array. Each pollinator entered the array and moved from plant to plant according to a sequence obtained by sampling from the set of pollinator movements observed in 2000. New pollinators continued to enter until the total number of foraging bouts equaled the number of bouts (18 for all pollinators) observed in the field. Second, to assess the impacts of ethological and mechanical isolation, we compared four types of simulations. Pollinators moved either at random between plants (no ethological isolation) or by sampling actual sequences (ethological isolation to the extent manifested in the observed visitation sequences). The probability of pollen deposition either was a fixed constant (no mechanical isolation) or was allowed to vary with the particular combination of type of donor flower and recipient flower (mechanical isolation to the extent manifested by actual differences in pollen deposition; Campbell et al. 1998). Finally, to evaluate the sensitivity of pollen transfer to yearly variation in pollinator behavior, we compared pollen transfer in simulations based on visitation in 2000 with that in simulations based on visitation to a similar mixed array of 21 plants in the summer of 1993 (data in Campbell et al. 1997). Simulations based on the 1993 data employed an array of 21 plants with visitation sequences sampled from the 32 hummingbird foraging bouts observed that year. Because number of flowers on a plant and proportion of flowers in pistillate phase differed between years, we also varied these aspects of floral display. In each case, output from the simulation was the average pollen transferred per recipient flower as a function of the type of donor plant and type of recipient plant. Because *Ipomopsis* is self-incompatible, we included only pollen transferred to flowers on other plants, just as we had in estimating pollen transfer with dyes in the experimental arrays. Each type of simulation was replicated 200 times.

Estimates of Parameter Values

All of the parameter values for simulations (tables 1, 2) were estimated from data obtained in this study or by Campbell et al. (1997, 1998). For each year of visitation data, the probability of visiting a flower was set equal to the slope of mean number of flowers visited on mean number of open flowers in a linear regression constrained

Table 2: Probability of pollen deposition used in the simulation model

Donor-recipient combination	Model 1	Model 2	Model 3
A-A	.135	.133	.120
A-H	.135	.111	.120
A-T	.135	.115	.120
H-A	.135	.082	.125
H-H	.135	.155	.125
H-T	.135	.142	.125
T-A	.135	.064	.064
T-H	.135	.226	.226
T-T	.135	.182	.182

Note: Probability of depositing pollen was estimated according to three models of pollen carryover: constant probability of deposition (model 1), probability allowed to vary for all donor-recipient combinations (model 2), and probability varied for *Ipomopsis tenuituba* donors only (model 3). A = *Ipomopsis aggregata*; H = hybrid; T = *I. tenuituba*.

Table 3: ANOVA table for the combined data from the 1999 and 2000 dye transfer experiments

Source of variation	df	MS	*F*	*P*
Recipient type	2	259.2	8.05	.0006
Donor type	2	158.2	4.91	.0094
Year	1	105.2	3.27	.0741
Recipient type × donor type	4	70.4	2.19	.0770
Recipient type × year	2	101.5	3.15	.0476
Donor type × year	2	27.9	.87	.4240
Three-way interaction	4	13.1	.41	.8037
Error	90	32.2		
A priori contrasts:				
Reproductive isolation:				
A donor to A vs. T	1	340.1	10.56	.0016
T donor to T vs. A	1	2.4	.07	.7868
Backcross symmetry	1	206.2	6.40	.0131

Note: A = *Ipomopsis aggregata*; H = hybrid; T = *Ipomopsis tenuituba*.

through the origin ($N = 83$, $R^2 = 0.85$ in 1993; $N = 27$, $R^2 = 0.87$ in 2000). Only plants that were visited were included in calculating probability of visiting a flower, since this parameter decides number of flowers visited once a pollinator has already approached a plant. The same probability of visitation was used for all plant types because ANCOVA revealed no interaction between the regression coefficient and plant type ($P = .70$ and $P = .14$ in the two years). Number of flowers displayed was based on average displays in our 1999 and 2000 arrays. The probability that a flower was pistillate rather than staminate was set to the mean value for proportion pistillate as described in Campbell (1989).

Two parameters, pollen pickup and probability of depositing pollen, were based on pollen transfer by captive hummingbirds (Campbell et al. 1998). In this earlier study (Campbell et al. 1998), hummingbirds picked up pollen from one of three types of donor flowers (*I. aggregata*, *I. tenuituba*, or natural hybrids) and then visited a series of 15 emasculated recipients containing flowers of all three types. Each bird completed one such sequence with each of the three donor types. We estimated pollen picked up at each staminate flower by comparing pollen remaining in the donor flowers visited once with pollen in unvisited flowers from the same plants.

In a situation with exponential pollen carryover, there is a constant probability of depositing a pollen grain. This probability can be estimated by regressing ln *Y* on the previous flower position ($n - 1$), where *Y* is the number of pollen grains deposited on the *n*th visit. The regression coefficient equals $\ln(1 - k)$, where *k* is the fraction of pollen removed from the pollinator at each visit (de Jong et al. 1992; Robertson 1992). We estimated the parameter *k* in three ways. First, we performed regression of ln *Y* using the combined data set and thus ignoring the identity of the donor or recipient flower (model 1 in table 2). This procedure gave a single constant for probability of depositing each pollen grain. It thus represented a situation of no mechanical isolation. Second, we allowed probability of pollen deposition to vary freely with the identity of donor and recipient by running a separate regression on data from each of the nine combinations (model 2 in table 2). This model encapsulates our best estimate of the degree of mechanical isolation. For the third model, we ran separate regressions for the three types of recipients of pollen originating at *I. tenuituba* donors only and combined recipients for the other types of donors (model 3). This model allows for mechanical isolation only when pollen originates at *I. tenuituba* and is justified by ANCOVA on ln *Y* showing an interaction between the effect of recipient type and previous flower position for *I. tenuituba* donors only ($P = .0024$). For pollen originating at an *I. tenuituba* donor, the probability of deposition on an *I. aggregata* stigma is only 35% as high as it is on a conspecific stigma (.064 vs. .182).

Results

Experimental Arrays

In the field arrays, dye receipt varied significantly with type of recipient and type of donor (table 3). In 1999, *Ipomopsis aggregata* received the most dye (recipient effect $F = 9.32$, df = 2, 45, $P < .0001$; fig. 1), while in 2000, hybrids received the most dye (recipient effect $F = 5.06$, df = 2, 45, $P = .0104$; fig. 2*A*). This led to a year × recipient interaction in the combined analysis (table 3). Perhaps the most striking pattern was that *Ipomopsis tenuituba*

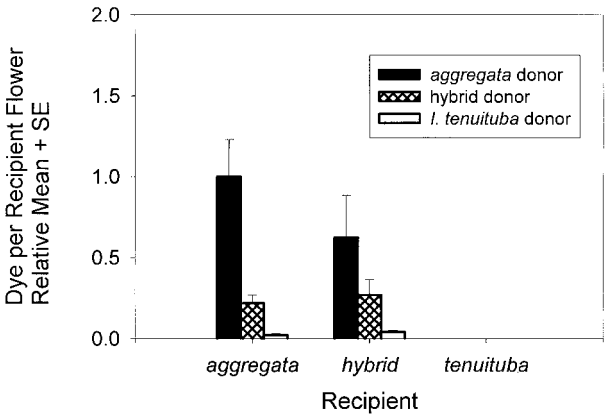


Figure 1: Mean dye transferred per recipient flower in the experimental array in 1999. To facilitate comparison with the simulations, we standardized values by dividing by the mean for the highest treatment. No dye was transferred to any of the *Ipomopsis tenuituba* flowers sampled.

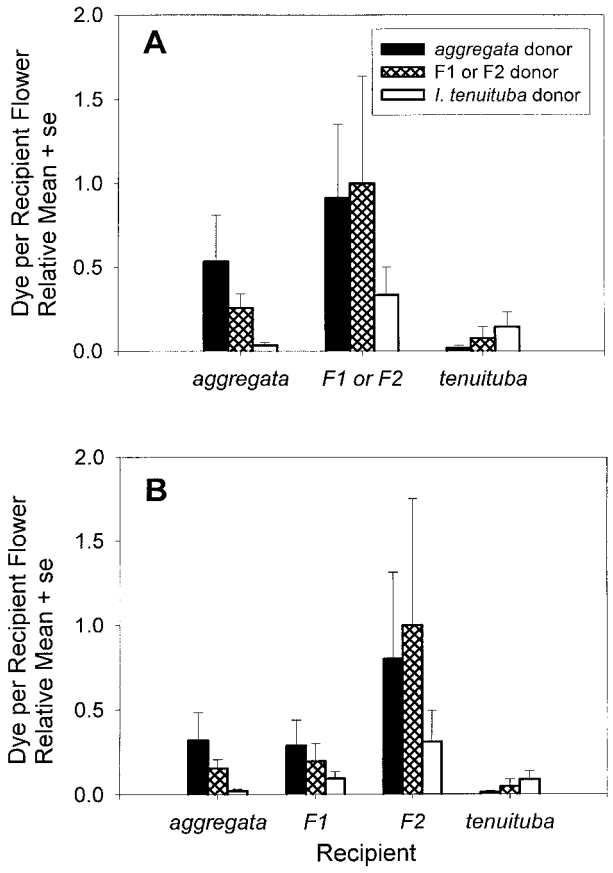


Figure 2: Mean dye transferred per recipient flower in the experimental array in 2000. *A*, F_1 and F_2 recipients combined into a single hybrid treatment. *B*, F_1 and F_2 recipients separated.

received very little dye (fig. 2) or even none (fig. 1). The type of hybrid strongly influenced dye receipt, as F_2 plants received significantly more dye than F_1 plants (contrast $F = 7.59$, df $= 1, 42$, $P = .0086$; fig. 2*B*). All types of plants set seed in response to natural fertilization during their time in the array.

More dye originating from *I. aggregata* reached stigmas of conspecific plants than reached stigmas of *I. tenuituba* (contrast $P = .0016$; figs. 1, 2). However, we detected no similar evidence for reproductive isolation when dye originated from *I. tenuituba* (contrast $P = .7868$; table 3). Even so, dye transfer indicating potential production of F_1 was low in this direction as well (figs. 1, 2), since there was so little dye dispersed from *I. tenuituba*. Starting at either *I. aggregata* or *I. tenuituba*, we found that, on average, only 7% as much dye was transferred to the alternate species as to a conspecific in 2000 and only 2% as much in 1999. In contrast with interspecific dye transfer, backcross transfer was high; in 2000, the overall rate of backcrossing between hybrids and one of the parental species actually exceeded conspecific dye transfer (fig. 2). This backcrossing was highly asymmetrical in absolute terms, with substantial transfer between hybrids and *I. aggregata* and much lower transfer between hybrids and *I. tenuituba* (contrast $P = .0131$; figs. 1, 2). On a relative basis, the amount of hybrid pollen received compared with conspecific pollen received was similar for the two species; however, F_1 and F_2 hybrids received only 37% as much from *I. tenuituba* as from *I. aggregata*.

Not only was average transfer between hybrids and *I. aggregata* high but also many stigmas received mixtures of these two types of pollen, as estimated by dye. In fact, the most common stigma load on *I. aggregata* flowers (23 of 51 stigmas with dye) was this two-way mixture (table 4). This mixture was 1.8 times as common as a pure load of dye originating from conspecific flowers. Mixtures of dye, especially the two-way mix of *I. aggregata* and hybrid and the three-way mixture, were also common on hybrid recipients (table 4).

We observed hummingbirds make 30 visits to *I. aggregata* plants, 39 visits to hybrid plants, and only five visits to *I. tenuituba* plants in our 2000 array. This ratio exhibits significant departure from equal preference ($\chi^2 = 25.16$, df $= 2$, $P < .001$). We saw no evidence that birds exhibited flower constancy to the species they started on, since the probability of moving to any given type of plant was independent of the type the bird last visited (contingency analysis, $G = 2.182$, df $= 4$, $P = .702$). Instead, birds made frequent transitions between plants of different types (fig. 3*A*). A bird that started at *I. aggregata* was most likely to fly to a hybrid and least likely to fly to *I. tenuituba*. This qualitative pattern was the same as the rank order of receipt of dye originating at *I. aggregata* (fig. 2*A*). The rank order for hummingbird visit sequences also matched the rank order for dye transfer when the bird started at a

Table 4: Frequencies of stigmas with various dye loads in the 1999 and 2000 arrays combined

Dye load	*Ipomopsis aggregata*	Hybrid	*Ipomopsis tenuituba*
None	39	64	90
Single type:			
Aggregata	13	6	0
Hybrid	7	8	0
Tenuituba	1	6	3
Mixture:			
Aggregata and hybrid	23	17	0
Aggregata and *tenuituba*	1	1	1
Hybrid and *tenuituba*	4	5	2
Aggregata, hybrid, and *tenuituba*	2	18	0

Note: In total, 311 stigmas were examined.

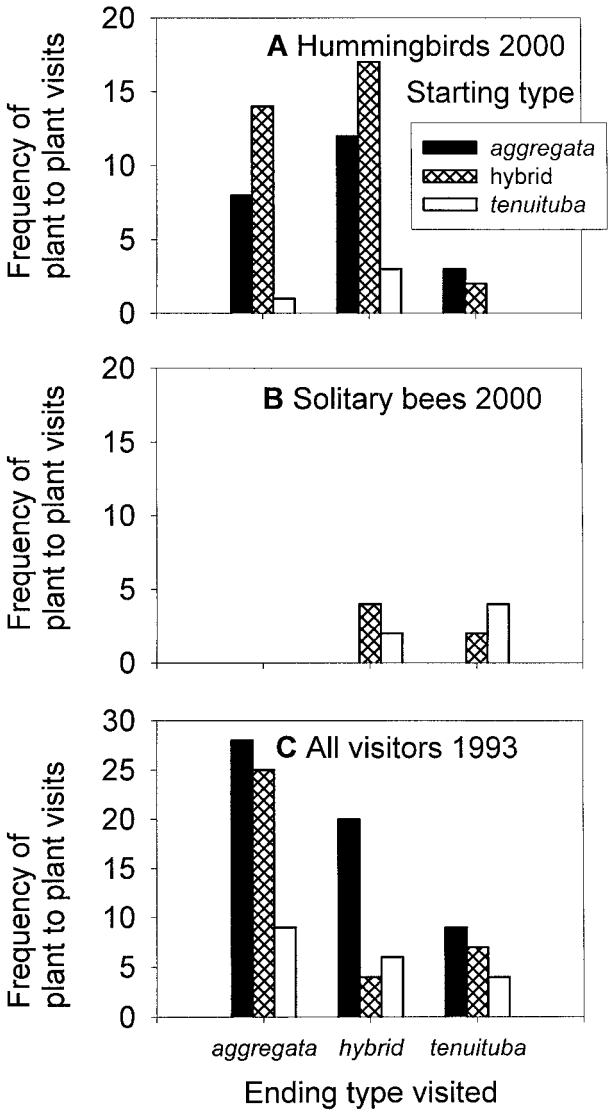


Figure 3: Frequency of plant-to-plant flights between the two species and hybrids. Some foraging bouts included visits to only one plant and are thus not displayed. *A*, Hummingbirds in 2000. *B*, Solitary bees in 2000. *C*, All visitors in 1993 (modified from Campbell et al. 1998).

hybrid (cf. figs. 2*A* and 3*A*). However, every bird that started at *I. tenuituba* flew to a different plant type, whereas we did note some conspecific *I. tenuituba* dye transfer. Combining flights that started at *I. aggregata* or *I. tenuituba*, we found that birds flew to the alternate species 50% as often as they flew to a conspecific plant. In contrast to hummingbirds, solitary *Andrena* bees flew only to hybrids and *I. tenuituba*, making eight plant visits to each (including cases in which only one plant in the array was visited), and consequently they made no interspecific flights during our observations. We observed four flights by bees between *I. tenuituba* plants (fig. 3*B*).

Per-flower visitation rates by hummingbirds in 2000 were highest to F_1 plants (ANOVA; $F = 4.57$, df $= 3, 32$, $P = .009$). Mean rates were 0.029, 0.047, 0.038, and 0.005 visits per flower per hour for *I. aggregata*, F_1, F_2, and *I. tenuituba*, respectively. This preference for hybrids contrasts with the preference we saw for *I. aggregata* in 1993, whereas the undervisitation of *I. tenuituba* was similar in both years (fig. 3*A*, 3*C*). In a multiple regression, visitation rate increased significantly with corolla width (slope = 0.047 visits/[flower × hour] per millimeter corolla width; $F = 6.23$, df $= 1, 21$, $P = .021$) but not with any of the other traits ($P > .05$), even though hummingbirds had responded to optical density as well as corolla width in 1993 (Campbell et al. 1997).

Simulation Model

Using floral display parameters and visit sequences from 2000 (table 1), the simulation model predicted highest pollen receipt for hybrid flowers and lowest for *I. tenuituba* (fig. 4*C*–4*F*). It also predicted higher backcrossing between hybrids and *I. aggregata* than between hybrids and *I. tenuituba*. These qualitative patterns resemble those we estimated with dyes in the actual 2000 array (fig. 2). The best fit to those estimates was obtained when we sampled from bee as well as bird visits and when we also allowed pollen carryover to depend on the specific combination of donor and recipient flowers (fig. 4*F*). Sampling of bee visitation sequences was necessary to predict any conspecific *I. tenuituba* transfer, since no bird in 2000 had been observed to visit more than one *I. tenuituba* plant before leaving the array.

These patterns of pollen transfer potentially reflect the influences of ethological and mechanical isolation. In simulations with random visitation sequences and constant carryover (fig. 4*A*), which therefore lacked either form of reproductive isolation, the mean rate of interspecific transfer was statistically equivalent to that of conspecific pollen transfer (109% as high in the 200 replicates). The difference in success among pollen donors in this null scenario was caused by variations in pollen production and flower number. Introducing ethological isolation by sampling observed hummingbird visitation sequences (fig. 4*C*) reduced the relative rate of interspecific pollen transfer to only 38% as high as conspecific transfer. In contrast, allowing for variable carryover and, hence, the influence of mechanical isolation (fig. 4*B*) reduced this figure only to 84%. Thus mechanical isolation, as reflected in the probabilities of depositing pollen in conspecific transitions versus interspecific transitions, had a smaller effect on simulation re-

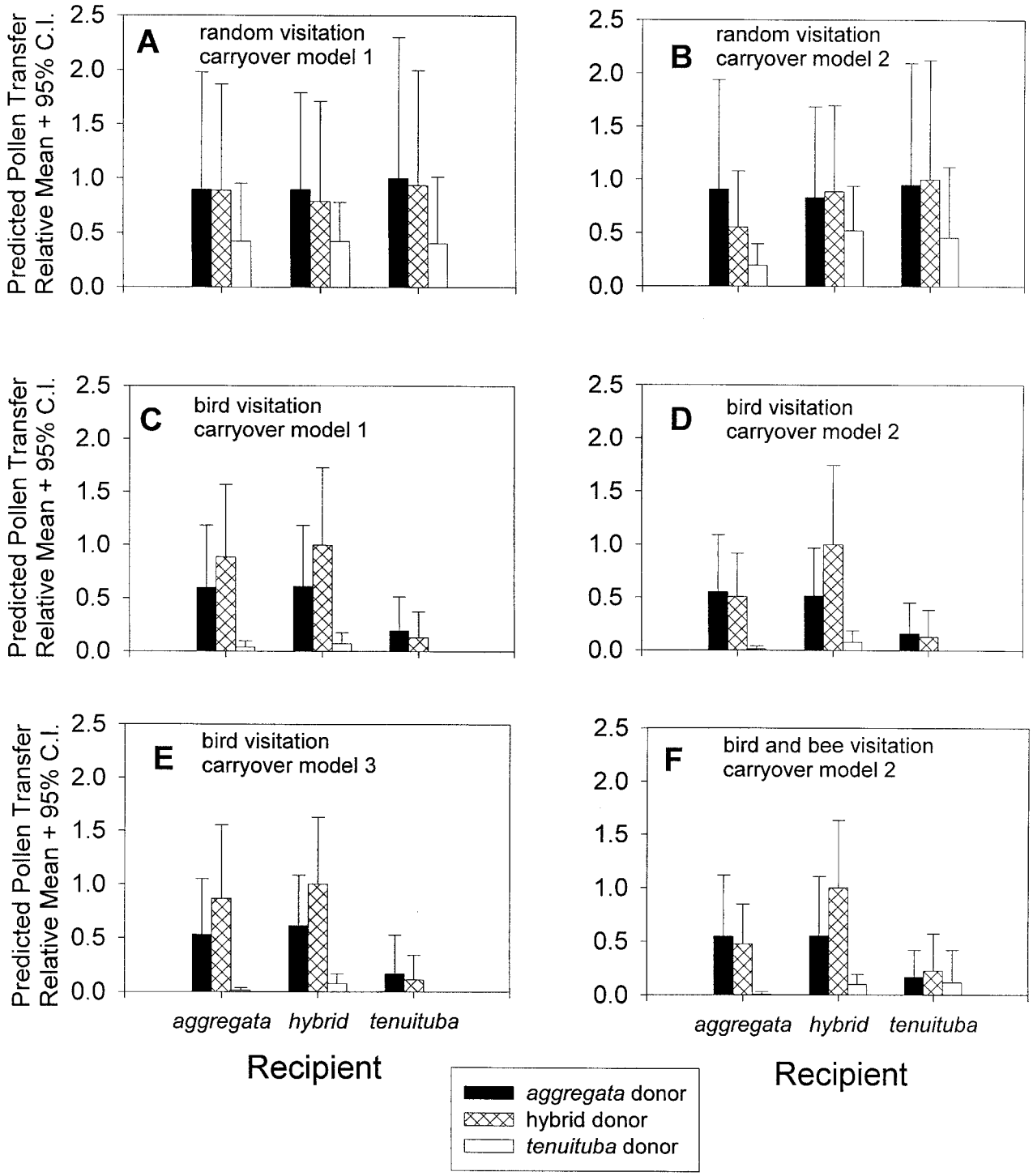


Figure 4: Effects of visitation behavior and variation in pollen deposition on predicted pollen transfer per flower in the 2000 array. Error bars show the ninety-fifth percentile based on 200 replicates of the simulation. All panels use floral displays from the 2000 array. *A*, Random visitation sequences, constant carryover (model 1 in table 2). *B*, Random visitation sequences, carryover varying with donor and recipient (model 2 in table 2). *C*, Observed hummingbird sequences sampled, model 1 carryover. *D*, Hummingbird sequences sampled, model 2 carryover. *E*, Hummingbird sequences sampled, carryover varying with recipient for *Ipomopsis tenuituba* donors (model 3 in table 2). *F*, Hummingbird and solitary bee sequences sampled, model 2 carryover.

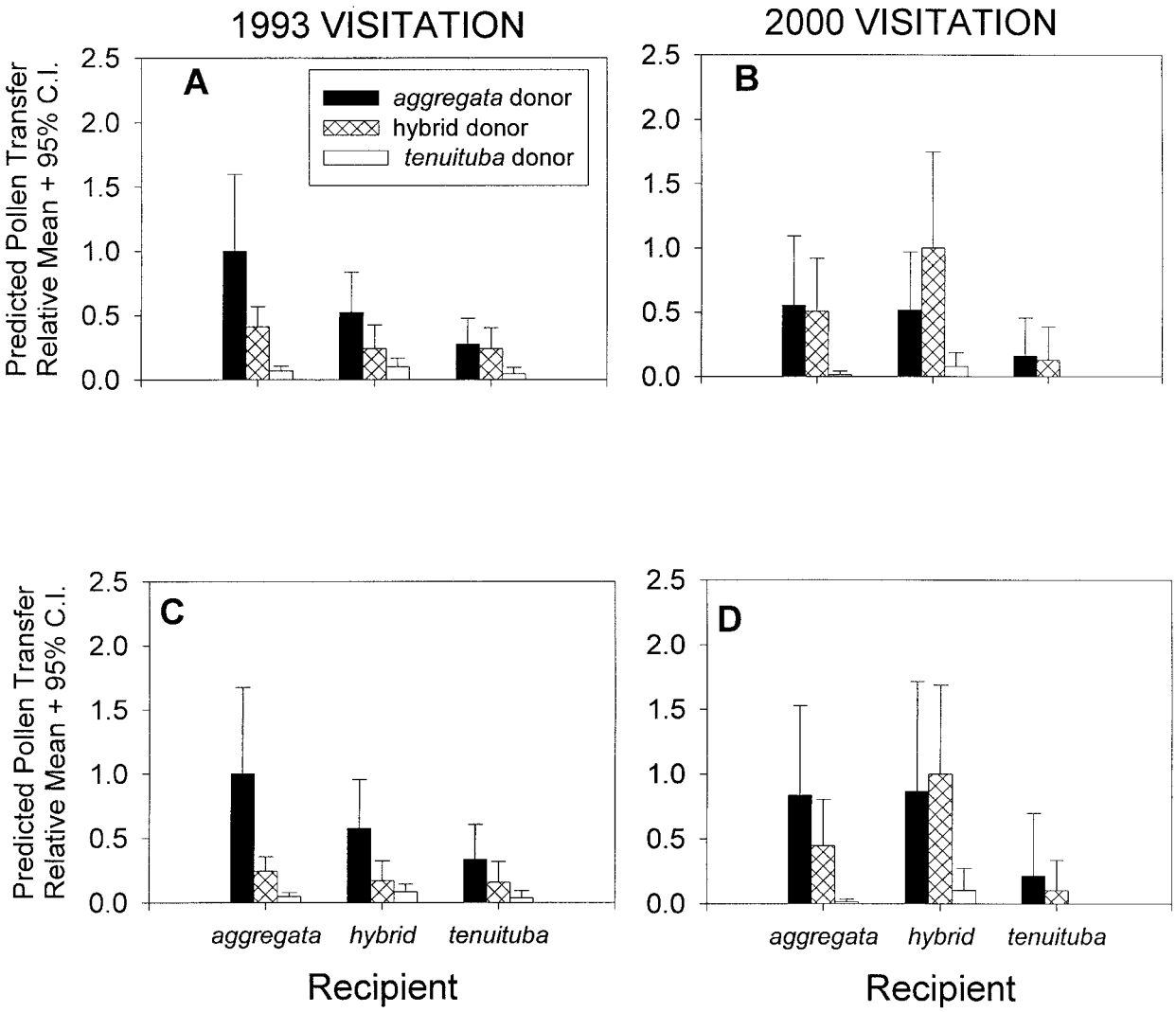


Figure 5: Effects of temporal variation in visitation and floral display on predicted pollen transfer per flower. Error bars show the ninety-fifth percentile based on 200 replicates of the simulation. Carryover varied with donor and recipient (model 2 carryover). *A*, 1993 visitation sequences sampled, 2000 floral display. *B*, 2000 hummingbird sequences sampled, 2000 floral display. *C*, 1993 visitation sequences sampled, 1999 floral display. *D*, 200 hummingbird sequences sampled, 1999 floral display.

sults than did ethological isolation. Nevertheless, adding mechanical isolation to the observed level of ethological isolation was necessary to explain how conspecific *I. aggregata* transfer could exceed that of hybrid to *I. aggregata* transfer (cf. fig. 4*D* and 4*C*), as we observed in the experimental array (fig. 2). At the same time, this addition of mechanical isolation reduced interspecific transfer only slightly from 38% to 31%, 33%, and 27% (fig. 4*D*–4*F*). All of these rates appear large compared to the observed value of 7% in the 2000 array, but there was considerable variation across replicates in this stochastic simulation, and 95% confidence intervals for these model outputs include the observed value from the 2000 array.

Additional evidence that pollen transfer is highly sensitive to visitation sequences comes from comparing the results of simulations that use visitation behaviors from different years (left vs. right panels in fig. 5). When we used visitation sequences from 1993, the preference of hummingbirds for *I. aggregata* (fig. 3*C*) always resulted in highest pollen transfer to that species. In contrast, the preference for hybrids in 2000 sometimes yielded higher pollen transfer to hybrids (fig. 5*B*). Thus year-to-year differences in visitation behavior altered the predicted relative pollination success of hybrids. In addition, interspecific pollen transfer that would generate an F_1 was slightly more common with the 1993 than with the 2000 visitation se-

quences (33% vs. 31% as high as conspecific transfer using floral displays from the 2000 array and 37% vs. 27% as high using floral displays from the 1999 array).

Discussion

By moving pollen, animal pollinators can influence both the rate of hybridization and the relative fitness of hybrids. Despite biologists' long recognition of this dual role of pollinators, few data exist on pollen transfer in natural hybrid zones. Here we have quantified these aspects of pollination patterns in an *Ipomopsis* hybrid zone. Hummingbird pollinators show strong, but not absolute, preference for flowers of *Ipomopsis aggregata* over those of *Ipomopsis tenuituba* (Campbell et al. 1997) and are capable of moving pollen between the species (Campbell et al. 1998), allowing hybridization to occur. Even so, pollen transfer between these species, estimated by dye transfer in experimental arrays, appeared to be relatively uncommon; the rate of interspecific transfer was 2%–7% as high as that of conspecific pollen transfer in spite of close intermixing of different plant types. In contrast, there was high potential for backcrossing; indeed, backcrossing from F_1 and F_2 hybrids to the parental species exceeded overall conspecific pollen transfer in the 2000 array. Potential backcrossing was less common with *I. tenuituba* than with *I. aggregata*, primarily because hummingbirds tend to avoid visiting the former species.

According to our simulation model, moderate levels of reproductive isolation in these arrays can be produced by the visitation behavior of pollinators, even without taking into account variation in pollinator effectiveness. This result suggests some ethological isolation brought about by the preference of hummingbirds for *I. aggregata*. This preference, in turn, results from the lower nectar production and narrower corolla tubes of *I. tenuituba* and association of these traits with white or pale pink rather than red flower color (Meléndez-Ackerman et al. 1997). In contrast, mechanical isolation appeared to cause little additional restriction to interspecific pollen transfer. Although birds are less likely to transfer *I. tenuituba* pollen when they visit an *I. aggregata* flower rather than another *I. tenuituba* flower, allowing for this sort of variation in pollinator effectiveness had only a slight impact on the rate of interspecific transfer in our simulations. Thus, in contrast to the suggestion of Grant (1993), our simulations suggest a weak role for mechanical isolation in this *Ipomopsis* system.

While these aspects of pollinator behavior predicted pollen transfer between species in experimental arrays, assessing the rate at which hybrids are formed in nature also requires information on relative seed sets, that is, on the postpollination component of reproductive isolation. In this hybrid zone, application of heterospecific pollen leads to as many seeds as does conspecific pollen (Campbell and Waser 2001), and even in competitive mixtures of pollen on the stigma, conspecific pollen has no advantage over heterospecific pollen in siring seeds (Alarcón and Campbell 2000). As a result, the rates of conspecific and interspecific dye transfer observed here should predict relative rates of pure and hybrid seed produced when plants of the two species are heavily intermixed, at least under similar pollinator conditions. Successful rates of backcrossing remain difficult to predict because the siring success of F_1 and F_2 hybrid pollen is still under study. Since a mixture of hybrid and *I. aggregata* pollen was the most common stigmatic load, it will be important to assess performance of hybrid pollen not only in single-donor pollinations but also in competition with *I. aggregata* pollen. Indeed, the preponderance of this kind of mixed load suggests a higher potential for pollen competition between hybrid and conspecific pollen than between pollen from the parental species. If these results are typical, investigators will need to go beyond the usual focus on mixtures of pollen from two species (reviewed in Arnold 1997) to characterize fully the importance of pollen competition.

Overall, hybrids exhibited relatively high pollination success, donating and receiving either more pollen than either parental species (2000) or an amount intermediate to those for the parents (1999). Pollination success is, of course, only one component of fitness. Hybrids must first survive to flowering, and once they receive pollen, they must go on to produce seed. We have shown elsewhere that *Ipomopsis* F_1 hybrids have survival as high as the average for the parental species and particularly high survival in certain environments (Campbell and Waser 2001). Moreover, they set abundant seed (Campbell and Waser 2001). In summary, the evidence so far suggests that these F_1 hybrids may be rarely formed in nature, but if formed, they have high fitness and are likely to leave backcross progeny. These results are consistent with Arnold's (1997) evolutionary novelty model, which proposes that hybrids rarely form under natural conditions but, once formed, can become established through positive exogenous selection.

First- and second-generation hybrids can differ in fitness since negative epistatic effects do not appear until the F_2 and later generations when new combinations of genes at different loci are formed (Lynch 1991; Fenster et al. 1997). With only three plants each of F_2 and F_1, we can provide only preliminary results on relative pollen receipt by different kinds of hybrids. However, we note that there was no evidence of hybrid breakdown in pollination success. Instead, the F_2 plants actually received more dye than expected in the absence of epistasis; their receipt exceeded

the average of the F_1 and midparental values (Lynch and Walsh 1997). This difference in dye receipt cannot be explained by greater attractiveness to pollinators, as F_2 plants did not receive a higher rate of pollinator visitation. Instead, it must relate to a higher effectiveness of pollen transfer when one of the F_2 plants was visited.

One of the more striking results of the study was the large difference in patterns of pollen transfer (as estimated by dyes) between the two years of investigation. We observed highest pollen receipt and, hence, a fitness advantage during pollination for *I. aggregata* in 1999, as we had previously predicted based on earlier studies of pollinator visitation (Campbell et al. 1997, 1998). However, in summer 2000, hybrids enjoyed the highest pollen receipt. Thus a relative fitness component for hybrids reversed between years. Pollinator behavior appears to be able to explain this change in fitness because the simulation model shows analogous reversals in pollen receipt depending on whether visitation is based on behavioral observations from 1993 or 2000. To a large extent, this reflects variation in behavior by the same pollinators, as hummingbirds preferred to visit *I. aggregata* in 1993 but made the most visits to hybrids in 2000. We suspect this change in behavior was a response to the use of potted F_1 and F_2 hybrids in 2000 instead of natural hybrids rather than some other difference in behavior over time. In other arrays involving natural hybrids, hummingbirds consistently preferred *I. aggregata* (Meléndez-Ackerman et al. 1997; Meléndez-Ackerman and Campbell 1998). Because the F_1 and F_2 plants were raised in pots at the RMBL while natural hybrids were raised in situ in the field, their differences in floral display (table 1), visitation, and pollination could be due to genetic differences or to differences in environmental conditions that altered attractiveness to pollinators.

Variation in composition of the pollinator fauna also contributed to the year-to-year differences. We observed visitation by *Andrena* bees only in summer 2000, and these visits appeared necessary to generate conspecific transfer of dye for *I. tenuituba* because we saw no flights between *I. tenuituba* plants by birds in that year. This suggests that solitary bees may be important pollinators of this species in occasional years, although even in their presence *I. tenuituba* had poor pollination success. Indeed, we have witnessed generally low fruit sets of this species at Poverty Gulch except in an unusual year when hawkmoths (*Hyles lineata*) were abundant (Campbell et al. 1997). Hawkmoths displayed a slight preference for narrow-tubed flowers, a trait characteristic of *I. tenuituba*, which could lead to different patterns of pollen transfer than those reported here. Furthermore, while hummingbirds are the only consistently common visitors to *Ipomopsis* at our study sites, in occasional years the plants are visited by swallowtail butterflies (*Papilio rutulus*) and bumblebees (*Bombus appositus*; Waser 1982). Such variation in pollinator behavior and composition may be common in other systems as well (Herrera 1988; Waser et al. 1996). If this sort of variation occurs in hybrid zones, it may lead to substantial temporal variation in the probability of hybrid formation and in hybrid fitness.

In conclusion, we found relatively low rates of interspecific pollen transfer but a remarkably high potential for backcrossing between hybrids and *I. aggregata*, with stigmas frequently receiving mixed loads of these two types of pollen. Our simulations suggest that the relatively low rate of interspecific pollen transfer was caused primarily by partial ethological isolation rather than by mechanical isolation. Indeed, patterns of pollen transfer in general were highly sensitive to the visitation patterns of pollinators, which differed across years. Pollen receipt by hybrids actually exceeded that of both parental species in one year and was intermediate in another year. These results suggest that natural variation in pollinator behavior and composition may lead to spatial or temporal differences in hybridization and fitness of hybrids. We urge other workers to examine mating patterns in other pairs of naturally hybridizing plant species and to do so over more than one year and site.

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