CONTEXT-DEPENDENT POLLINATOR BEHAVIOR: AN EXPLANATION FOR PATTERNS OF HYBRIDIZATION AMONG THREE SPECIES OF INDIAN PAINTBRUSH

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In some areas of sympatry, reproductively compatible plant species hybridize, but in other areas of sympatry, they do not and they remain reproductively isolated from one another. Explanations offered to explain patterns of hybridization that vary by population have usually focused on genetic or environmental factors. Instead, we examined whether different community contexts might change pollinator preference and constancy and thus influence the likelihood of hybridization among three Indian paintbrush species (*Castilleja miniata*, *C. rhexifolia,* and *C. sulphurea*). To determine whether visitation was context-dependent, we observed pollinator behavior in experimental arrays (constructed using flowering stems of the three Indian paintbrush species) in different contexts. Contexts were defined by which *Castilleja* species occurred in the immediate neighborhood of the arrays. Specifically, we asked, does visitation to particular species in the arrays depend on context? In general, each *Castilleja* species was preferred when it matched the surrounding community context, as is predicted by optimal foraging theory. More interestingly, pollinator constancy was weakened in the hybrid context (an area where the three species co-occurred with morphologically intermediate plants), which is likely to increase pollen flow among the species. Reduced pollinator constancy in hybrid zones could set up a positive feedback loop in which more flower diversity is created through hybridization, decreasing pollinator constancy, and leading to more hybridization. This self-reinforcing mechanism could lead to "hybridization hot spots" and to a patchy distribution of hybrid populations. We expect that this mechanism may be important in other animal-pollinated plant hybrid zones.

KEY WORDS: *Castilleja,* constancy, facilitation, hybridization, preference.

Hybridization is usually studied with an eye toward understanding reproductive isolation between species, but an emerging view is that hybridization, especially in plants, may play a creative role in generating variation that leads to diversification among populations and communities. It has even been suggested that the majority of plant species are products of past interspecific hybridization events (Grant 1981; Ellstrand et al. 1996; Rieseberg 1997). Although interspecific hybridization may be common in plant lineages, a pair of reproductively compatible species may hybridize in one area of sympatric contact but remain reproduc-

tively isolated in another area (Harrison 1986; Rieseberg et al. 1988; Grant 1993; Arnold 1997). Explanations for spatial variation in hybridization have mostly focused on genetic factors (e.g., parental allelic or ploidy-level incompatibilities; Stebbins and Tobgy 1944; Brochmann et al. 1992; Young 1996), plant physiological traits (e.g., differences in photosynthesis; Johnston et al. 2001), or water use efficiency (Fischer et al. 2004)), and/or abiotic attributes of the environment (e.g., differences in habitat requirements; Harrison 1986; Cruzan and Arnold 1993; Arnold 1997; Emms and Arnold 1997; Goulson and Jerrim 1997; Nagy

1997; Johnston et al. 2001). Biotic factors, such as herbivores, pollinators and plant competitors, could be equally important, but they have received less attention (Grant 1952, 1993; Chase and Raven 1975; Jones 1978; Campbell et al. 1997; Goulson and Jerrim 1997; Melendez-Ackerman et al. 1997; Wesselingh and Arnold 2000). The aim of our study was to determine whether differential pollinator behavior could explain the patchy patterns of hybridization observed among three species of Indian paintbrushes (*Castilleja*, Orobanchaceae).

In plants, the formation of F_1 hybrids first requires that pollen from one species be transferred to the stigma of another species. Thus, for animal-pollinated plants, the initial stages of hybridization will be influenced by pollinator movement patterns (Campbell et al. 1997; Wesselingh and Arnold 2000), which can have several effects on hybrid zone dynamics. First, pollinator preferences for particular plant species (Cock 1978) can lead to disproportionate fitness payoffs among some plants, if the more strongly preferred plants experience increased pollen receipt or seed output (e.g., female fitness) and/or increased pollen donation or siring success (e.g., male fitness). Extreme pollinator preferences for particular flower morphs of the same species can also create reproductive barriers between morphs that may ultimately lead to speciation (Jones 1978; Grant 1993, 1994). Second, patterns of interspecific hybridization can also be influenced by pollinator constancy, which is defined as the degree to which an individual pollinator specializes on a particular flower species while skipping alternative species that offer equivalent rewards during a foraging bout (Waser 1986). A constant pollinator moves nonrandomly and promotes assortative mating within a particular species, whereas an inconstant pollinator promotes interspecific pollen transfer that could ultimately lead to the formation of hybrids, if postzygotic reproductive barriers are incomplete. Nonrandom foraging among plant species has been observed for many pollinators (Waser 1986; Chittka et al. 1999; Jones and Reithel 2001). Although pollinator constancy usually refers to situations where the rewards (i.e., pollen or nectar) of alternative plant options are equivalent (Waser 1986), similar evolutionary outcomes are likely when plant rewards are not exactly equal but the species co-occur in mingled populations.

In areas of sympatry, both plant-specific traits and ecological attributes of the environment can affect pollinator movement patterns between species. Floral rewards, such as pollen and nectar, are important attractants to pollinators, and the quantity and quality of these rewards can influence pollinator visitation rates and plant mating success (Zimmerman and Pyke 1988; Robertson et al. 1999; Utelli and Roy 2000). In addition, specific traits that communicate reward quantity or quality to pollinators (e.g., petal and bract color), and plant traits that affect pollinator access to rewards (e.g., flower corolla tube length and width) can be highly variable within hybrid zones (Inouye et al. 1980; Waser and

Price 1981; Grant and Temeles 1992; Melendez-Ackerman et al. 1997; Wesselingh and Arnold 2000). These differences among plant species could serve as the basis for pollinator discrimination (Grant 1993, 1994). Furthermore, in areas of sympatry, reproductive isolation among closely related plant species may arise from differences in the timing of flower production (i.e., ethological reproductive isolation; Waser 1978a, 1978b). Pollinator visitation patterns can also be influenced by ecological attributes of plant populations and communities. For example, pollinator activity and the abundance and diversity of the pollinator fauna can vary markedly with differences in plant population density (Webb and Bawa 1983; Kwak 1987; Bosch and Waser 1999, 2001), population size (Klinkhamer and de Jong 1990; Jennersten and Nilsson 1993), plant community composition (Thomson 1978; Moeller 2004; Moeller and Geber 2005), site geography (Eckert 2002), microclimate (Klinkhamer et al. 1989), elevation (Scobell and Scott 2002), or across years and seasons (Herrera 1988, 1989; Roy 1996; Bosch and Waser 2001).

The three species we examined, *Castilleja miniata*, *C. rhexifolia*, and *C. sulphurea*, commonly co-occur across numerous localities within the Rocky Mountains of North America. In some areas of sympatry, presumed hybridization has obscured morphological distinctions between these three species, whereas in other locations these species do not appear to hybridize and remain phenotypically distinct (Heckard and Chaung 1977; E. Hersch, pers. obs.). We addressed the following four questions: (1) do different pollinator species show distinct preference patterns for the three *Castilleja* species? (2) could pollinator behavioral patterns promote pollen flow between the species (are pollinators constant)?, (3) do the species vary in nectar traits (quantity and quality) and, if so, do differences among species correlate with pollinator preference and constancy patterns? and (4) are pollinator movement patterns (defined by preference and constancy behaviors) shaped by the surrounding *Castilleja* community context? We expected that pollinator activity would vary with the community context because pollinators are predicted to forage economically by maximizing their energy gain per unit time invested in foraging (Stephens and Krebs 1986). Optimal foraging could either be accomplished by choosing plants that offer higher rewards and/or by reducing floral handling or search times. If pollinators in this system forage optimally, we predicted that pollinators would have stronger preferences for plant species when they were most represented in the surrounding *Castilleja* community context, as this should minimize searching and handling times.

Materials and Methods
STUDY SYSTEM

The three species studied, *C. miniata*, *C. rhexifolia,* and *C. sulphurea,* are perennial, nearly self-incompatible (E. Hersch,
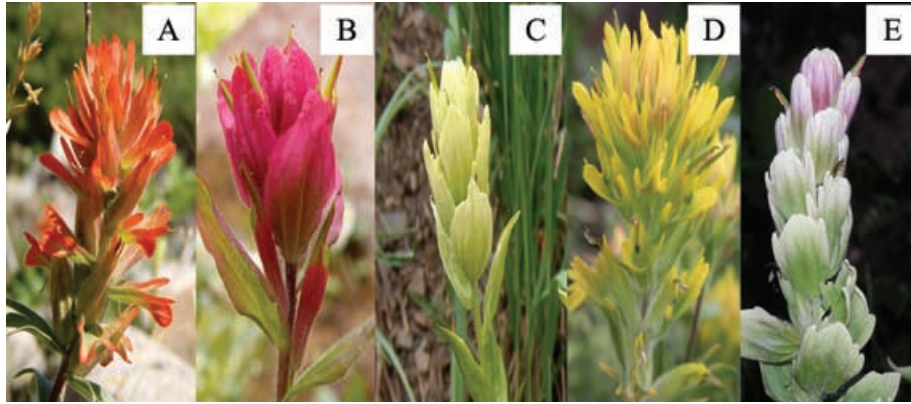
Figure 1. The three *Castilleja* species that were used in the pollinator experimental arrays: (A) *C. miniata*, (B) *C. rhexifolia*, and (C) *C. sulphurea*, plus some examples of hybrid individuals of unknown origin (D and E).

unpubl. data) hemiparasitic herbs that commonly co-occur where we did this study, in the Rocky Mountains of western Colorado. All three species have small inconspicuous flowers surrounded by showy, brightly colored bracts; *C. miniata* has red, *C. rhexifolia* has pink, and *C. sulphurea* has yellow floral bracts and hybrids have bracts that combine the colors of yellow, pink, and red (Fig. 1). In the area of this study, the flowering periods of these three species overlap and plants continuously produce flowers from late June to mid August (E. Hersch, pers. obs.). Because these three species occur and flower in sympatry, are largely self-incompatible, and have several traits characteristic of animal-pollinated plants, it seems likely that patterns of pollinator behavior could influence patterns of pollen transfer between these species.

POLLINATOR VISITATION PATTERNS

Pollinator activity was observed in experimental arrays during July and August of 2003 and 2004 for a total of 18 and 30 h, respectively. The setup of the experimental arrays was the same in both years (Fig. 2). Arrays consisted of four subarrays that were arranged in a square with the diagonal separating subarrays equal to 7 m. Each subarray consisted of nine freshly picked *Castilleja* flowering stalks (racemes) arranged in floral aquapics (small plastic vases, Syndicate Sales, Inc., Kokomo, IN) in a 3 × 3 grid. Three of the subarrays consisted of nine racemes of a single species, while the fourth array contained three racemes of each species. No naturally growing *Castilleja* plants were located within any of the four subarrays, although some naturally occurring *Castilleja* plants grew in the space separating the subarrays. All four subarrays were simultaneously observed during each 45-min replicate and the number of pollinator visits to racemes, pollinator identity, and visit duration to the nearest second were recorded. In 2004, the number of pollinator movements to individual flowers within

racemes was also recorded. Pollinators were recorded as visiting a raceme or flower when they approached a plant and proceeded to probe a flower, making contact with the stigma and/or pollen possible. Visit duration was recorded for visitors only when they were in contact with the sexual parts of flowers, not when they were basking or resting on other plant parts.

To determine whether the surrounding plant community influenced pollinator behavior, experimental arrays were set up in four distinct contexts (defined by which *Castilleja* species were present within a 20-m radius of the arrays); the red context had *C. miniata*, the pink context had *C. rhexifolia*, and the yellow
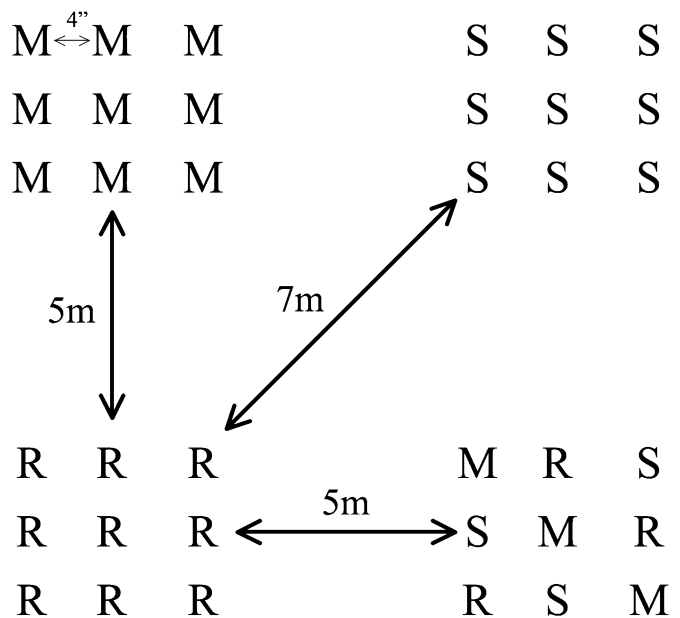

Figure 2. Design of experimental arrays. Letters represent racemes of the three *Castilleja* species: M, *C. miniata*; R, *C. rhexifolia*; and S, *C. sulphurea*.

context had *C. sulphurea* plants in the immediate vicinity. In 2004, experimental arrays were also set up in an area where the three *Castilleja* species co-occurred with morphologically intermediate plants (herein referred to as the hybrid context). All experimental arrays were set up in montane meadows near the Rocky Mountain Biological Laboratory, Gunnison County, CO; the red, pink, and yellow contexts were near Schofield Pass ($\approx$3465–3535 m elevation) and the hybrid context was located along the trail leading to Copper Lake ($\approx$ 3500 m elevation). Because hybrids were not used in any of the experimental arrays, data from this study indicate only how pollinators might affect the formation of F_1 hybrids, but not the subsequent formation of backcross hybrids.

All observations were made under clear to partly cloudy skies between 0800 h and 1700 h, which is when the majority of pollinators visited *Castilleja* spp. (E. Hersch and A. Fong, unpubl. data). Racemes were collected the day before they were used in the experimental arrays and were stored in water at 4°C. No flowering stems that showed visible signs of pest damage were used in the arrays and flowering stems that became wilted during the experiments were replaced. The number of replicates observed in a single day depended on the weather and ranged between one and four replicates per day. To eliminate any biases, subarrays, flowers within the subarrays, and observers were randomized between each replicate.

Preference patterns

In 64 replicates, we observed 987 (446 in 2003 and 541 in 2004) separate pollinator foraging bouts (instances where a pollinator entered an array and visited at least one raceme), which accounted for 1842 pollinator visits to racemes (1065 in 2003 and 777 in 2004). Each pollinator was identified as nearly as possible to species, and was easily classified into one of five different pollinator groups: bumblebees (*Bombus* spp.), flies (including members of Muscidae, Anthomyiidae, and Tachinidae), hoverflies (mostly members of Syrphidae, but also some Bombyliidae), hummingbirds (*Selasphorus platycercus* and *S. rufus*), and "other." The "other" category was dropped from subsequent analyses because it was not composed of a coherent group of pollinators and it also accounted for a low percentage of the total observed visits (5.8% of all visits from both years of the study). To examine pollinator preference patterns, we analyzed the data with three-way fixed-effect analyses of covariance (ANCOVAs) on the mean number of raceme visits in a replicate (square-root transformed) with the factors of recipient plant (*C. rhexifolia*, *C. miniata*, and *C. sulphurea*), pollinator group (bumblebee, fly, hoverfly, and hummingbird), context (red, yellow, pink, and hybrid [2004 only]), and their interaction terms. Date within a year was used as a covariate in the analyses because pollinator abundances and visitation rates can vary across different days in a season (Sih and Baltus 1987; Rathcke 1988). We analyzed the results separately for each year of the

study because we added a new context in the second year, and an initial model that included year revealed significant interactions with year.

Different contexts may have different pollinator assemblages due to differences in the availability of specific plants preferred by particular pollinator groups. For the 2004 data, which included the hybrid context, we used an a priori orthogonal contrast to test whether, on average, experimental racemes in the hybrid context received more visits than experimental racemes placed in the other, more uniform contexts. We contrasted the number of pollinator visits in the hybrid context against the average number of pollinator visits from the other three contexts. We also used a priori orthogonal contrasts to test whether any observed differences in visitation rates to the species between contexts in a given year could be attributed to the probability of similar plants being found in the immediate vicinity. We contrasted the number of visits to plants matching the array context (*C. miniata* in the red and hybrid contexts, *C. rhexifolia* in the pink and hybrid contexts, and *C. sulphurea* in the yellow and hybrid contexts) with the number of visits to plants not matching the array context (*C. miniata* in the yellow and pink contexts, *C. rhexifolia* in the yellow and red contexts, and *C. sulphurea* in the red and pink contexts).

Intraraceme movements

Geitonogamy is a form of selfing in which pollen is moved between different flowers within a single plant or raceme (Richards 1997). For nearly self-incompatible plants, geitonogamy can be detrimental because it can lead to reductions in fitness. From the 2004 pollination visitation data, we calculated the number of flowers visited per raceme visit (total racemes visited = 425). To examine the potential for geitonogamous mating, we analyzed the data with a three-way fixed-effect analysis of variance (ANOVA) on the average number of flowers visited per raceme visit (square-root transformed) with the factors of pollinator group, recipient plant, context, and their interaction terms. Small sample sizes of visits by certain pollinator groups in some contexts prevented analysis of the three-way interaction and this higher-order interaction was dropped from the final model. Date of observation was originally used as a covariate in the analysis, but because it was not significant ($P = 0.83$), it was dropped from the model to increase power. Differences in the number of flowers visited per raceme visit among the three plant species could be attributed to species differences in the number of open flowers per raceme. To examine this possibility, we counted the number of open flowers on all racemes used in the experimental arrays and we tested for differences among the plant species using a univariate ANOVA.

Visit duration patterns

Quality of pollinator visitation can be negatively or positively correlated with visit duration. For example, pollinators that spend

more time visiting a plant could be moving between multiple flowers within a plant, thus promoting geitonogamous mating or limiting pollen export. Alternatively, pollinators that spend more time visiting plants may gather more pollen that can then be exported to other plants (Mitchell and Waser 1992). One measure of pollinator visit duration is the average time spent per raceme visit. To examine whether pollinators spent, on average, similar amounts of time visiting racemes, and whether the species identity of the recipient raceme influenced visit duration, we used two-way fixed-effect ANCOVAs on the average raceme visit duration (log transformed) with the factors of pollinator group, recipient plant, and their interaction. As above, date was used as a covariate in the analyses and the data were analyzed separately for each year of the study, because an additional context was added in the second year. Using the 2004 data, we addressed whether differences in raceme visit duration patterns were likely due to differences in the number of flowers visited per raceme visit and/or to differences in the duration of flower visits. First, we looked at the correlation between the total number of flowers visited (square-root transformed) and the total visit duration (log-transformed). Second, we used a two-way fixed-effect ANCOVA on the average flower visit duration (log transformed) to examine whether pollinators spent, on average, similar amounts of time visiting individual flowers on the racemes, and whether the species identity of the recipient plant influenced flower visit duration. As above, pollinator group, recipient plant, and their interaction terms were factors in the analysis and date was used as a covariate.

INTERSPECIES MOVEMENT

To determine whether pollinator behavior could promote interspecific pollen transfer, we followed individual pollinators that visited the "mixed" subarrays and we counted the total number of transitions made between racemes of the same and of different plant species summed across the two years of the study. Using this transition matrix, we calculated Bateman's constancy indices ("B.I.," see Table 3; Bateman 1951; Waser 1986). Because this index compares pollinator transitions between two species at a time (Bateman 1951; Waser 1986), indices for each pollinator group were calculated separately for each of the three possible species transition pairs: movements between *C. rhexifolia* and *C. sulphurea* (R+S), between *C. miniata* and *C. sulphurea* (M+S), and between *C. rhexifolia* and *C. miniata* (R+M).

We did not observe enough transitions in each context (total observed transitions were 29, 47, 85, and 38 in the red, pink, yellow, and hybrid contexts, respectively) to have sufficient power to calculate Bateman's indices separately for each context by pollinator group by species transition pair. However, we could test whether context influenced the overall degree of pollinator constancy and whether the movement patterns of the different pollinator groups depended on the community context. To do this,

we used a two-way fixed-effect ANOVA on the percentage of between-species transitions out of the total number of transitions observed in each foraging bout with the factors of context, pollinator group, and their interaction term. Data were analyzed separately for each year because the hybrid context was added to the study design in the second year.

FLORAL NECTAR REWARDS

We analyzed nectar to determine the degree to which pollinator preferences were correlated with differences in nectar quality or quantity. Nectar extraction in *Castilleja* spp. is tricky because the corolla tube is so narrow, and it usually results in damage to the flower from which the nectar was extracted. Therefore, instead of measuring nectar directly from the flowers that were used in the experimental arrays, we measured the quantity and quality of standing nectar (the nectar found in a flower at any given time not accounting for the possibility of prior visits by pollinators or nectar robbers) from 15 random flowers per species in 2003 and from 30 random flowers per species in 2004. All plants from which nectar was measured naturally occurred at one of the four contexts in which the experimental arrays were set up. Nectar quantities and sugar concentrations were measured using standard procedures (see Kearns and Inouye 1993b). The number of open flowers per raceme was counted to examine whether the total number of open flowers affects nectar traits. Prior to setting up the experimental arrays, we verified that neither the time of day that nectar was extracted nor cutting significantly affected nectar production.

Three-way fixed-effect ANCOVAs were performed on nectar volume (log transformed) and sugar concentration with the factors of year (2003 or 2004), plant species (*C. miniata*, *C. rhexifolia*, and *C. sulphurea*), treatment (cut or standing), and their interaction terms. Nectar volume and sugar concentration data were not highly correlated (Pearson's $r = -0.148$), and were thus analyzed with separate univariate ANCOVAs. The number of open flowers per raceme was used as a covariate in the analyses.

In all analyses, response variables were transformed when necessary to satisfy assumptions of normality and homogeneity of variances (Sokal and Rohlf 2000). Tukey's HSD tests were used for a posteriori comparisons among means. Data are presented as LS means $\pm$ 1 standard error (SE). All analyses were performed using JMP-version 5.1.2 statistical package (SAS 2001).

Results
DO POLLINATORS SHOW DISTINCT PREFERENCE PATTERNS?
Preference patterns
Visitation to racemes differed between the two years of the study and more pollinator visits were made to experimental arrays in 2003 than in 2004 (mean numbers of raceme visits per replicate

Table 1. Analyses of covariance (ANCOVAs) on pollinator visitation for (a) 2003 and (b) 2004.

Source of variation	df	SS	F	P
A 2003 – Raceme visitation				
Context	2	30.12	18.30	<0.0001
Pollinator group	3	27.66	11.14	<0.0001
Recipient plant	2	35.80	21.75	<0.0001
Context × Pollinator group	6	23.84	4.83	0.0001
Context × Recipient plant	4	25.29	7.68	<0.0001
Recipient plant × Pollinator group	6	66.70	13.51	<0.0001
Three-way interaction	12	55.58	5.63	<0.0001
Date	1	18.73	22.76	<0.0001
Error	251	478.54		
B 2004 – Raceme visitation				
Context	3	4.97	5.45	0.0011
Pollinator group	3	29.76	32.65	<0.0001
Recipient plant	2	11.42	18.80	<0.0001
Context × Pollinator group	9	21.84	7.99	<0.0001
Context × Recipient plant	6	15.22	8.35	<0.0001
Recipient plant × Pollinator group	6	31.68	17.38	<0.0001
Three-way interaction	18	15.60	2.85	<0.0001
Date	1	0.83	2.73	0.0993
Error	431	130.93		

Pollinator visitation to racemes was measured separately for each pollinator group as the number of visits to a *Castilleja* species per 45-min replicate (24 in 2003, 40 in 2004). Date of observation was used as a covariate in the analyses.

Table 2. Pollinator visitation patterns observed in the experimental arrays for different recipient plant species, pollinator groups, and contexts for the two years of study.

Factor	Raceme visitation 2003	Raceme visitation 2004	Intraraceme movement 2004
Recipient plant			
C. miniata	1.30±0.56	0.68±0.17	1.55±0.11
C. rhexifolia	4.83±0.56	1.53±0.17	1.52±0.08
C. sulphurea	4.62±0.56	2.18±0.17	1.19±0.16
Pollinator group			
Bumblebee	6.63±0.64	1.77±0.20	1.64±0.12
Fly	3.47±0.64	2.85±0.20	1.23±0.09
Hoverfly	2.40±0.64	0.59±0.20	1.22±0.11
Hummingbird	1.83±0.64	0.65±0.20	1.59±0.20
Context			
Red	1.45±0.57	0.83±0.20	1.37±0.12
Pink	2.42±0.56	1.97±0.20	1.29±0.12
Yellow	6.87±0.57	1.41±0.14	1.72±0.11
Hybrid		1.64±0.17	1.30±0.11

Raceme visitation was measured as the number of pollinator visits per raceme in a 45-min replicate. Intraraceme movements (2004 only) were measured as the number of flowers visited per raceme visit in a 45-min replicate. Raw data are presented as treatment least squares (LS) means ± 1 standard error (SE). Data were transformed for analyses. Within a response variable, different superscript letters following LS means for each factor indicate significant differences (*P* ≤ 0.05) among means shown by Tukey's HSD posteriori tests.

were 49.14 and 17.58 for 2003 and 2004, respectively). Within each year, visitation rates depended on the recipient plant species identity, the pollinator group, and the context in which the experimental arrays were set up (Table 1). In 2003, the species with pink bracts, *C. rhexifolia*, received significantly more visits than the species with yellow bracts, *C. sulphurea,* and both species received significantly more visits than the species with red bracts, *C. miniata*, while in 2004, *C. sulphurea* received significantly more visits than *C. rhexifolia* and both species received significantly more visits than *C. miniata* (Table 2). In both years, we observed the fewest number of visits to racemes in the red context. However, in 2003, we observed the most number of raceme visits in the yellow context, whereas in 2004, we observed the most number of raceme visits in the pink and the hybrid contexts (Table 2). Differences among contexts in the total number of raceme visits were not entirely due to the probability of similar plant species occurring in the immediate vicinity (contrast of total visits in the hybrid context against the mean number of visits in the other three contexts for the 2004 data: $F_{1,431} = 1.76$, $P = 0.1857$). There were also differences between the two years in the relative abundances of the different pollinator groups observed visiting the arrays. In 2003,

bumblebees made significantly more visits to the arrays than any of the other three pollinator groups, whereas in 2004, flies made the most visits to the arrays (Table 2). In 2003 (as indicated by the significance of the date covariate), but not in 2004, we observed more visits to racemes as the season progressed (Table 1).

Perhaps the most striking pattern observed in both years was that visitation to racemes of each recipient plant species was significantly affected by the array context and the pollinator group identity (Table 1). As predicted by optimal foraging theory, plant species received more visits when they matched the context (contrast of mean number of visits to recipient species matching the array context against the mean number of visits to recipient species not matching the array context for 2003: $F_{1,251} = 28.77$, $P < 0.001$, and for 2004: $F_{1,431} = 38.93$, $P < 0.001$). For example, in both years *C. sulphurea* received the most visits in the yellow context and *C. rhexifolia* received the most visits in the pink context (Fig. 3 A, D). However, the strength of this finding varied between years and for each species by context pair (e.g., compare visitation data for *C. miniata* across years, Fig. 3 A, D). In general, bumblebees made more visits to *C. sulphurea* racemes, hummingbirds made more visits to *C. miniata* racemes, and both
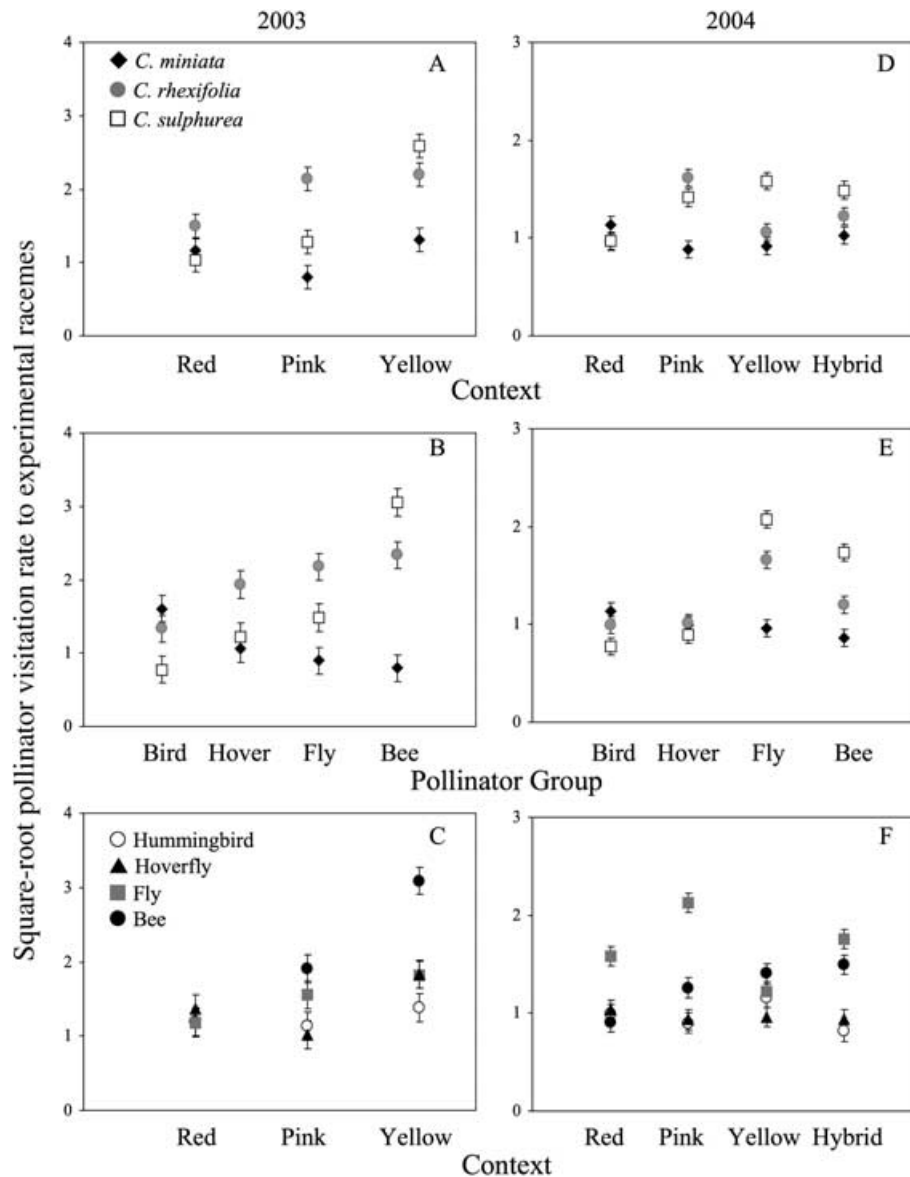
Figure 3. Least-square means $\pm$ 1 standard error (SE) for observed pollinator visitation rates to racemes (measured as the square-root of the number of pollinator visits per raceme per 45-min replicate) in relation to recipient plant species, pollinator group, and array context for (A–C) 2003 and (D–F) 2004.

of these two pollinator groups made intermediate numbers of visits to *C. rhexifolia* racemes (Fig. 3 B, E). However, fly and hoverfly visitation patterns to the three *Castilleja* species differed between years (compare fly and hoverfly visitation patterns between years in Fig. 3 B and E).

The context of the experimental arrays also influenced the number of raceme visits made by the different pollinator groups (Table 1). In both years, hummingbirds made more visits to the arrays in the red and yellow contexts and bumblebees made more visits to the arrays in the yellow and hybrid contexts (Fig. 3 C and F). Fly visitation patterns, however, changed dramatically between years. In 2003, fly raceme visitation rates did not significantly

differ between contexts (Fig. 3 C), whereas in 2004, flies made more visits to racemes in the pink and hybrid contexts (Fig. 3 F).

Intraraceme movements

The context of the experimental arrays also influenced the number of intraraceme movements ($F_{3,200} = 3.89$, $P = 0.0101$). Pollinators visited the most number of flowers per raceme visit in the yellow context and the least number of flowers per raceme visit in the pink and hybrid contexts (Table 2). Contextual differences in the number of flowers visited per raceme visit make sense in light of significant differences among pollinator groups in the number
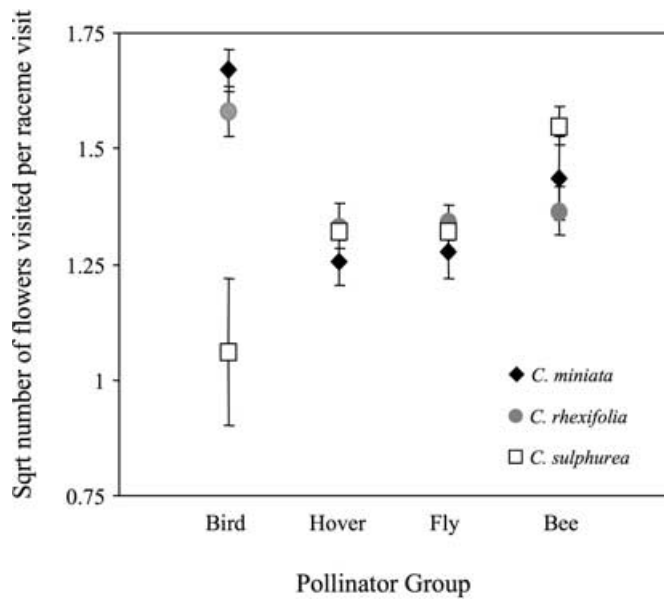
Figure 4. Least-square means ± 1 standard error (SE) for the average number of flowers visited per raceme visit (square-root transformed) made by the different pollinator groups when visiting racemes of the three *Castilleja* species.

of intraraceme movements ($F_{3,200} = 5.06$, $P = 0.0022$) and in the overall visitation patterns observed. For example, bumblebees ($\mu = 1.64$) tended to visit more flowers per raceme visit than hummingbirds ($\mu = 1.59$), and they both visited significantly more flowers per raceme visit than flies ($\mu = 1.23$) and hoverflies ($\mu = 1.22$; Table 2). Furthermore, observations in the yellow context accounted for a large proportion of the total raceme visits made

by bumblebees, while observations in both the red and the yellow contexts accounted for a large proportion of the total raceme visits made by hummingbirds (Fig. 3 F).

Racemes of the three plant species used in the experimental arrays had different numbers of open flowers: *C. miniata* racemes had significantly more open flowers ($\mu = 5.9 \pm 0.08$) than *C. sulphurea* ($\mu = 5.2 \pm 0.08$) racemes, which had significantly more open flowers than *C. rhexifolia* ($\mu = 4.9 \pm 0.08$) racemes ($F_{2,1434} = 39.91$, $P < 0.0001$). However, despite such differences, the three recipient plant species did not differ in the number of intraraceme visits that they received ($F_{2,200} = 1.96$, $P = 0.1442$, Table 2). The average number of flowers visited per raceme visit by the different pollinator groups did, however, depend on the species identity of the recipient plant (significant pollinator group by recipient plant species interaction, $F_{6,200} = 3.89$, $P = 0.0011$). Bumblebees made more intraraceme movements when foraging on *C. sulphurea* individuals and hummingbirds made more intraraceme movements when foraging on *C. miniata* and to a lesser extent *C. rhexifolia* racemes (Fig. 4). In contrast, flies and hoverflies visited similar numbers of flowers per raceme visit regardless of the plant species being visited (Fig. 4). The number of intraraceme visits to the different recipient plant species or made by the different pollinator groups did not significantly depend on context.

Visit duration patterns

In both years of the study, pollinator groups significantly differed in the average amount of time they spent visiting racemes (2003: $F_{3,134} = 17.47$, $P < 0.0001$; 2004: $F_{3,200} = 11.61$, $P = 0.0004$). In
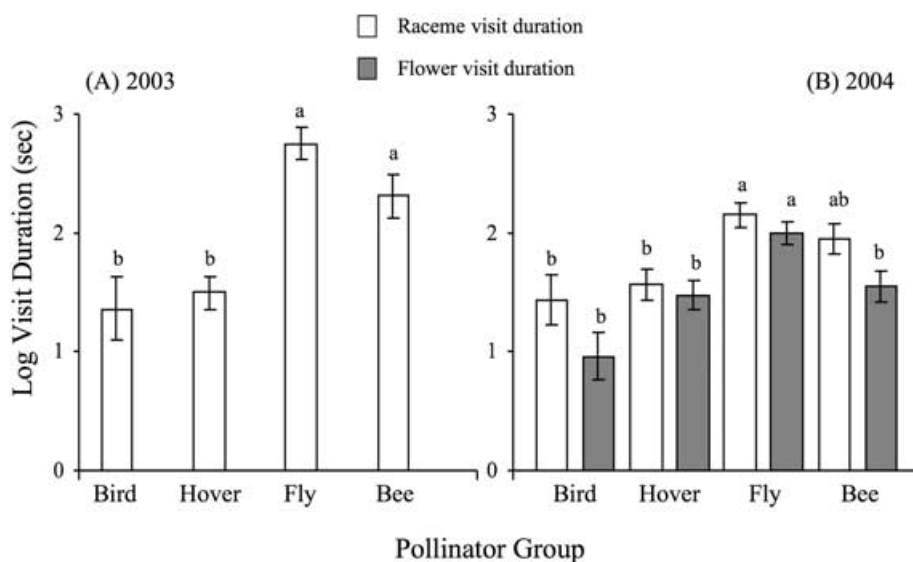

Figure 5. Average visit duration (log transformed) to racemes and flowers made by different pollinator groups in (A) 2003 and (B) 2004. Bars represent least-square means $\pm$ 1 standard error (SE). Different lower case letters indicate significant differences between factor levels using the Tukey's HSD test.

Table 3. Contingency table showing the number of transitions made by different pollinator groups from one raceme (listed horizontally) to another raceme (listed vertically) summed across both years of the study. Racemes are identified by species: M = *C. miniata*, R = *C. rhexifolia*, and S = *C. sulphurea*. Data from this contingency table were used to calculate Bateman's constancy indices (after Waser 1986). Bateman's constancy index (B.I.) is calculated as: B.I. = ($\sqrt{AD}$ – $\sqrt{BC}$) / ($\sqrt{AD}$ + $\sqrt{BC}$), where A and D are the number of within-species transitions for species 1 to species 1 (A) and for species 2 to species 2 (D), and B and C are the number of between-species transitions from species 1 to species 2 (B) and from species 2 to species 1 (C). Bateman's constancy indices vary between–1, in which case pollinator visitation is perfectly dissassortative, and +1 in which case pollinator visitation is perfectly assortative. Index values near zero suggest that a pollinator visits the different plant species at random.

			Hummingbird			Hoverfly			Fly			Bumblebee		
From			M	R	S	M	R	S	M	R	S	M	R	S
To		M	7	8	1	3	1	1	1	1	1	1	0	0
		R	6	7	0	3	13	5	1	11	13	1	13	17
		S	1	1	1	0	9	2	0	8	8	0	17	37

general, flies made the longest raceme visits, followed by bumble-bees, and then hoverflies, and hummingbirds (Fig. 5 A, B). Some of this variance in raceme visit duration might be attributed to differences in the number of flowers visited. For example, as pollinators visited more flowers, they generally spent more time foraging (for all pollinator groups combined, $R^2 = 0.56, P < 0.0001$; Fig. 6). Also, differences among pollinator groups in the average raceme visit duration are likely due to significant differences among pollinator groups in the time they spent visiting individual flowers on a raceme ($F_{3,200} = 14.80, P < 0.0001$). In general, flies made the longest flower visits, followed by bumblebees, and then hoverflies, and hummingbirds (Fig. 5 B). Visit duration patterns to racemes (in both years) and to individual flowers (in 2004) did not differ among the recipient plant species visited, nor was there a significant pollinator group by recipient plant interaction. As the season progressed in 2004, pollinators spent more time visiting racemes ($F_{1,200} = 10.63, P = 0.0013$) and flowers ($F_{1,200} = 12.41, P = 0.0005$).

COULD POLLINATOR BEHAVIOR PROMOTE INTERSPECIES POLLEN MOVEMENT?

Across both years, we observed a total of 199 pollinator transitions between racemes within the "mixed" subarrays, with 48% of these transitions occurring between racemes of different species (see Table 3). Of the total number of transitions observed to each species in a pair, pollinators made more interspecific movements between *C. rhexifolia* and *C. sulphurea* (76.1%) than between *C. rhexifolia* and *C. miniata* (37.5%) and between *C. miniata* and *C. sulphurea* (6.7%). Pollinator groups differed in their degree of constancy, and bumblebees, flies, and hoverflies were more constant when flying between *C. miniata* and *C. rhexifolia* (B.I. = 1.00, 0.54, 0.57, respectively) or between *C. miniata* and *C. sulphurea* (B.I. = 1.00, 1.00, 1.00, respectively), than when flying between *C. rhexifolia* and *C. sulphurea* (B.I. = 0.13, −0.04, −0.14, respectively). In contrast, hummingbirds were more constant when flying between *C. sulphurea* and *C. rhexifolia*

(B.I. = 1.0) and between *C. sulphurea* and *C. miniata* (B.I. = 0.45), than when flying between *C. rhexifolia* and *C. miniata* (B.I. = 0.01).

The percentage of between-species transitions significantly differed between contexts in 2004 ($F_{3,35} = 12.71, P < 0.0001$), but not in 2003 ($F_{2,53} = 0.05, P = 0.9554$). In 2004, more between-species transitions were made within experimental arrays in the hybrid context (83.3 ± 0.1) than within experimental arrays in the red (14.6 ± 0.1), pink (51.7 ± 0.1), or yellow (14.3 ± 0.1) contexts. Also, in 2004 ($F_{3,35} = 3.21, P = 0.0347$), but not in 2003 ($F_{3,53} = 0.13, P = 0.9396$), the percentage of between-species transitions differed among pollinator groups. Hummingbirds (68.5 ± 9.5) made more between-species transitions than
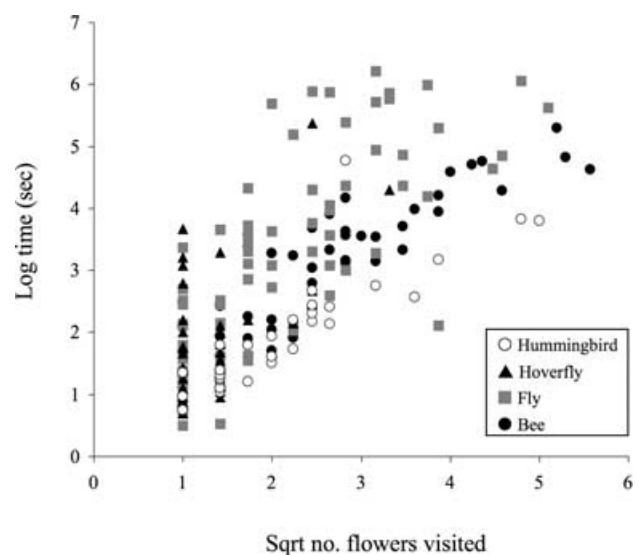

Figure 6. The relation between the total time spent foraging (log transformed) and the total number of flowers visited (square-root transformed) for the different pollinator groups: bumblebees (•; $R^2 = 0.77, P < 0.0001$), flies (▪; $R^2 = 0.57, P < 0.0001$), hoverflies (▲; $R^2 = 0.27, P = 0.0004$), and hummingbirds (○; $R^2 = 0.74, P < 0.0001$).
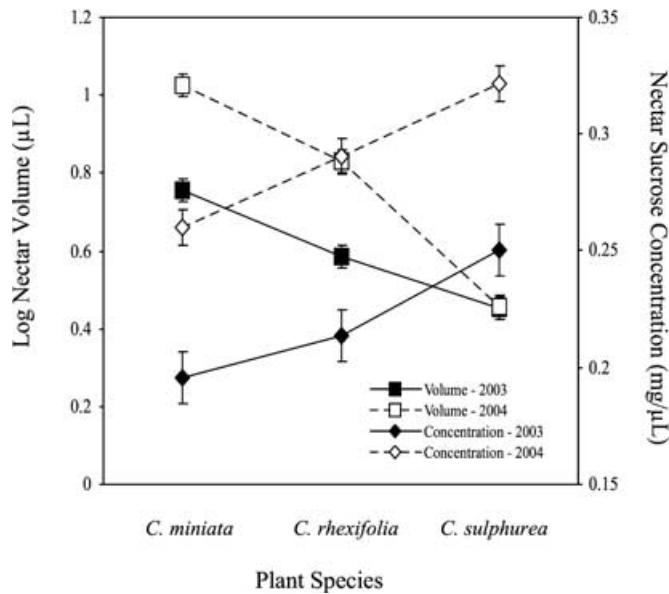
Figure 7. Least-square means $\pm$ 1 standard error (SE) for nectar volume (log μL) and nectar sucrose concentration (mg/μL) for the three *Castilleja* species measured from flowers in 2003 and in 2004.

hoverflies (35.4 $\pm$ 0.1), flies (33.3 $\pm$ 0.1), or bumblebees (26.7 $\pm$ 0.1). In neither year did the percentage of between-species transitions made by the different pollinator groups depend on the context in which the experimental arrays were set up.

DO THE SPECIES OFFER SIMILAR NECTAR REWARDS?

Nectar sugar concentration and nectar volume differed among the species ($F_{1,287} = 80.2$, $P < 0.0001$). *Castilleja miniata* had the highest volumes of the least concentrated nectar, *C. sulphurea* had the lowest volumes of the most concentrated nectar, and *C. rhexifolia* had intermediate values of these two nectar traits (Fig. 7). Both nectar concentration ($F_{1,287} = 80.2$, $P < 0.0001$) and nectar volume ($F_{1,287} = 23.8$, $P < 0.0001$) were greater in 2004 than in 2003, although there was a significant species*year interaction for nectar volume ($F_{2,287} = 5.9$, $P = 0.0032$). For *C. sulphurea*, measured nectar volume did not increase in 2004 over 2003, as it did for the other two species (Fig. 7). The number of open flowers on a raceme did not significantly affect standing nectar volume or sugar concentration.

Discussion

For closely related, sympatric, animal-pollinated plants, patterns of hybridization and the fitness differences among plants should depend on pollinator visitation patterns, the spatial and temporal variability of pollinators, the pollen transfer efficiencies of different pollinators, and the probability of pollination leading to successful production of viable offspring. Here we found that pollinators have distinct preferences for particular species, but that

the local community context significantly influenced pollinator behavioral patterns. We also found that one species, *Castilleja rhexifolia*, might facilitate pollinator movement between species in this system, because it was commonly visited by both hummingbirds (which normally preferred *C. miniata*) and bumblebees (which normally preferred *C. sulphurea*). Furthermore, advanced stages of hybridization may be accelerated in hybrid zones or areas where the three species co-occur as mingled populations, because pollinator constancy was weakened in these regions.

POLLINATOR PREFERENCE PATTERNS
AND PLANT FITNESS

Pollinators showed distinct preferences for particular *Castilleja* species. In both years of the study, hummingbirds made more visits to *C. miniata* and *C. rhexifolia* racemes than to *C. sulphurea* racemes, and bumblebees made more visits to *C. sulphurea* and *C. rhexifolia* racemes than to *C. miniata* racemes. *Castilleja miniata* has red floral bracts and flowers characterized by high volumes of dilute nectar, *C. sulphurea* has yellow floral bracts and flowers characterized by low volumes of more concentrated nectar, and *C. rhexifolia* has pink floral bracts and flowers characterized by intermediate volumes of moderately concentrated nectar. The preference patterns and differences in plant traits we found are in agreement with other studies, which have shown that hummingbirds are often attracted to plants that have reddish floral displays (Grant 1966; Duffield 1971; Melendez-Ackerman et al. 1997) and higher volumes of less concentrated nectar than the yellow and blue flowers of bumblebee-pollinated plants (Pyke and Waser 1981; Thomson et al. 2000). Hummingbirds only forage for nectar, but bumblebees forage for both nectar and pollen and their foraging behavior can be modified by pollen availability (Cresswell and Robertson 1994; Rasheed and Harder 1997 a, b). Naturally occurring *C. sulphurea* and *C. rhexifolia* flowers produce more pollen than *C. miniata* flowers (E. Hersch, unpubl. data), which may have influenced bumblebee foraging patterns.

Not all of the observed pollination patterns were static across years. For example, fly and hoverfly visitation rates to the three *Castilleja* species varied among the two years of the study. In 2003, both flies and hoverflies made more visits to *C. rhexifolia* racemes, whereas in 2004, flies made more visits to *C. sulphurea* racemes and hoverflies did not discriminate among the three *Castilleja* species. In general, fly preference patterns were consistent with other studies, which have shown that flies often prefer lighter colored flowers (Faegri and van der Pijl 1980; Johnson et al. 2002), particularly yellow ones (Kevan 1972, 1983; Vernon and Borden 1983; Vargas et al. 1991). The relative abundances of raceme visits made by the different pollinator groups in each context also varied among years. In 2003, bumblebees were the most common pollinators observed in the pink and yellow contexts, but in 2004,

flies were the most common pollinators observed in the majority of contexts, except for the yellow context, in which bumblebees were the most common pollinators. It is not known exactly why pollinator preference patterns change between years and sites (see, for e.g., Herrera 1988; Waser et al. 1996; Campbell 2002). However, fluctuations in weather conditions across years and between sites could directly and indirectly (e.g., through changes in plant traits or phenology) contribute to variable pollinator activity. Between the two field seasons, some trees fell at the sites and this could have altered microsite differences among the contexts. Also, there were significant differences in weather patterns across years, with 2003 being drier and hotter than 2004. In cooler conditions, flies tend to be more common, or more willing to fly than bees and hummingbirds (Kearns 1990, 1992; Kearns and Inouye 1994; Larson et al. 2001). This might help to explain why flies were relatively more abundant visitors in 2004.

Pollinator visitation rates are only one component of a pollinator's service (Herrera 1987; Waser and Price 1990; Inouye et al. 1994), and pollinator preference patterns for particular *Castilleja* species may or may not result in variable plant mating success. We did not measure the effectiveness of pollen transfer by the different pollinator species and/or resulting seed production, but it can partially be inferred from studies that have examined pollinator effectiveness for flowers having similar morphologies. Waser and colleagues (Waser 1978a; Mayfield et al. 2001) have found that hummingbirds and bumblebees are effective pollinators for other co-occurring plants in this region, like *Delphinium nelsoni* and *Ipomopsis aggregata*, which have flowers with long corollas like the *Castilleja* species studied here. Thus, when they are foraging, hummingbirds and bumblebees may be very effective at transferring pollen between *Castilleja* flowers, as large pollen loads were observed on individuals in both groups (E. Hersch, pers. obs.). Flies were also observed gathering pollen (but not nectar) of the *Castilleja* flowers that they visited (E. Hersch, pers. obs.). Thus, under cooler conditions when hummingbirds and bumblebees are less common, flies may be extremely important pollinators because they are present (Kearns 1992; Kearns and Inouye 1993a), can carry pollen between species (Kearns 1992; Erhardt 1993; Roy 1996), and, if they visit more frequently, they can deposit as much or more pollen on flowers as bumblebees and hummingbirds (Kearns and Inouye 1994). Nonetheless, without measuring pollen deposition, we cannot be certain that visitation patterns actually translate into beneficial pollen receipt. For example, we found that bumblebees and hummingbirds tended to visit more flowers on racemes of the plant species that they preferred (e.g., *C. sulphurea* and *C. miniata*, respectively). Because all three *Castilleja* species are partially self-incompatible (E. Hersch, unpubl. data), such a pattern where bumblebees and hummingbirds visit more flowers per raceme could actually decrease plant fitness by increasing the probability of geitonogamy. Interestingly, flies and hoverflies

tended to visit fewer flowers per raceme than bumblebees and hummingbirds.

Finally, we do not know how differences in visit duration patterns influence plant fitness. Pollinator groups differed in the average amount of time they spent visiting racemes, but pollinator groups did not spend different amounts of time visiting racemes of the three plant species. Although differences among pollinator groups in the duration of raceme visits could partially be explained by differences in the average number of flowers visited per raceme visit, pollinators also spent different amounts of time visiting individual flowers on the racemes. Another study found that the number of visits made by bumblebees was positively correlated with the number of pollen grains received by a plant, but that pollinator visit duration was the best predictor of pollen export (Jones and Reithel 2001). In 2004, we found that flies were the most abundant visitors and that they also spent the longest time visiting flowers. If flies are effective pollinators for *Castilleja* plants, then in cooler years, flies might significantly increase both female (pollen receipt) and male (pollen donation) plant fitness, while at the same time limiting the chances for geitonomous mating because they switch racemes more often. Differences in the mechanisms employed by pollinators when foraging could also influence visit duration patterns. For example, bumblebees gather pollen and carry it away to feed their young, hummingbirds go for nectar and indirectly pick up pollen, whereas flies often rest on the sexual parts of plants as they consume pollen. To best understand how pollinator preference and visit duration patterns actually translate into differences in plant fitness, future studies should examine the efficacy of specific pollinators in this system.

CONTEXT-DEPENDENT POLLINATOR PREFERENCE PATTERNS

Pollinator preference patterns were not fixed and often varied with the community context. In general, *Castilleja* species tended to receive more visits when they matched the surrounding community context, which makes sense if pollinators are foraging optimally (Krebs 1978; Stephens and Krebs 1986). For example, *C. sulphurea* received the majority of the visits observed in the yellow context and *C. rhexifolia* received the majority of the visits observed in the pink context. Emms and Arnold (2000) examined visitation rates to *Iris fulva*, *I. hexagona,* and their naturally occurring hybrids and found that the parental species in the experimental arrays that matched those that were naturally occurring in the area also received more visits. From the "pollinator's perspective," optimal foraging theory predicts that pollinators should focus on the most prevalent plant species at any given time. From the "plant's perspective," facilitation of pollinators could occur when co-occurring plants (both conspecific and heterospecific plants) mutually attract pollinators (Hollings 1959; Thomson 1978, 1981; Brown and Kodric-Brown 1979; Roy and

Widmer 1999) or jointly maintain populations of resident pollinators (Hollings 1959; Schemske 1981; Moeller 2004). From both a pollinator and a plant perspective, we predicted that higher visitation rates to racemes would be observed in the hybrid context because this community contained both representatives of each of the three species and hybrids. However, we did not find that visitation to racemes was increased in the hybrid context. These results are similar to those by Campbell et al. (1997), who observed lower visitation rates when experimental arrays were placed in an *Ipomopsis* hybrid zone than when they were placed in areas dominated by one of the parental species. The fact that two studies have found results different than predicted suggests that pollinators may be cuing into something that we do not yet understand. One idea to test in the future is that they are responding to color density or frequency.

POLLINATOR CONSTANCY AND THE POTENTIAL FOR HYBRIDIZATION

Pollinator preference patterns were reinforced by patterns of pollinator constancy because pollinators tended to fly either between racemes of the same species or between racemes of different *Castilleja* species in the order of preference. For example, hummingbirds were more likely to fly between racemes of *C. miniata* and *C. rhexifolia* than from these species to *C. sulphurea*. Bumblebees, on the other hand, were more likely to fly between racemes of *C. sulphurea* and *C. rhexifolia* than between racemes of either of these species and *C. miniata*. These findings suggest that prezygotic barriers to hybridization via pollination are leaky and furthermore that *C. rhexifolia* might facilitate pollinator-mediated hybridization in this system. For example, initial stages of hybridization via bumblebee foraging may occur among *C. sulphurea* and *C. rhexifolia* plants, whereas initial stages of hybridization via hummingbird foraging may occur among *C. miniata* and *C. rhexifolia* plants. Flies and hoverflies were also less constant when flying between *C. sulphurea* and *C. rhexifolia* racemes and these pollinators could also promote hybridization among *Castilleja* species. All three *Castilleja* species are able to produce F_1 hybrid seeds when hand pollinated with pollen from one of the other two *Castilleja* species (unpubl. ms., E. I. Hersch); thus, pollinator movement patterns between the species are likely to lead to F_1 hybrid formation.

We found that the proportion of between-species transfers made by pollinators depended on the community context in which the experimental arrays were placed. Specifically, more between-species transfers (less constant pollinators) were observed in the hybrid context than in any of the other three contexts. Leebens-Mack and Milligan (1998) found that hybrid plants in experimental arrays act like "bridges," facilitating pollinator foraging between the parental species. This suggests that once the rare initial stages of hybridization are established in an area (e.g., the cre-

ation of F_1 hybrid plants), these areas can then act as hybridization hot spots in which more advanced stages of hybridization proceed at accelerated rates because pollinators are less constant in these areas. Although this hypothesis is not directly tested with the data gathered here, it is tentatively supported by the observation that hybridization among these three species is patchy, but that when hybridization does occur, it seems to occur over relatively broad areas. Context-dependent pollinator preference and constancy patterns may be important in establishing and maintaining the patchy distribution of hybrid zones observed among other animal-pollinating angiosperms.

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