

SPATIOTEMPORAL VARIATION IN THE ROLE OF HUMMINGBIRDS AS POLLINATORS OF *SILENE VIRGINICA*

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Abstract. Pollination restricted to a guild of visitors is central to the concept of pollination syndromes. However, there is limited quantitative evidence that the pollination of plants exhibiting certain pollination syndromes is actually dominated by the expected guild of pollinators. We determined the importance of the sole hummingbird species in eastern North America, the Ruby-throat, *Archilochus colubris*, and invertebrate visitors as pollinators of *Silene virginica*, which exhibits traits commonly associated with hummingbird pollination. We quantified the role of either type of visitor as pollinators by comparing female reproductive success of plants that were allowed free access by all visitors to female reproductive success of caged plants that were restricted to pollination by invertebrates (small bees and flies, and in one year *Bombus* spp.). Spatial and temporal variation in the role of either type of pollinator was documented by comparing the female reproductive success of caged and control plants in two nearby sites for five years. In general we found that hummingbirds are the predominant pollinator of *S. virginica*, but there is considerable spatiotemporal variation in their role. We discuss the importance of understanding the role of hummingbirds as potential selective agents of *S. virginica* floral traits within the greater community context of other potential pollinators.

Key words: *Archilochus colubris*; Fire-pink; hummingbirds; plant–animal interactions; pollination; pollination syndromes; *Silene virginica*.

INTRODUCTION

One of the most studied of plant–animal interactions is that between plants and their pollinators (Sprengel 1793; Darwin 1859, 1862, Muller 1883, Knuth 1908, 1909, Baker 1963, Grant and Grant 1965, Faegri and Van der Pijl 1979, Thompson 1994). It is not difficult to understand why the evolution of pollination systems has been under such long and intense scrutiny. The evolution of floral form has mostly been used in the past to define plant species and it is floral form that determines the pollination system. Thus the evolution of pollination systems may be concordant with the evolution of species, or at the very least, reproductive isolation (Baker 1963, Bradshaw et al. 1995). Evolutionary trends were recognized early on for flower morphology (Bessey 1915, Stebbins 1970) which in turn were also implicated in the evolution of increasing pollinator specialization and plant diversification (Grant and Grant 1965, Stebbins 1970, 1974). There is evidence that plant diversification is associated with animal pollination (Eriksson and Bremer 1992) and pollinator specialization (Armbruster 1992, 1993, Hodges and Arnold 1994). Consequently, it is thought that flowers having traits representing particular pollination syndromes reflect the action of long-term natural selection by particular guilds of pollinators or pollinators which are functionally equivalent in their size and be-

havior (Waser 1983, Waser et al. 1996, Armbruster et al. 2000).

However, the utility of pollination syndromes has long been questioned (Robertson 1928) and has been recently critically reexamined (Waser et al. 1996, Olertson 1996, 1998). As a first step in quantifying whether uniform selection pressures have led to the convergent evolution of floral traits representing pollination syndromes, Waser et al. (1996) suggest experimental approaches that include documenting the visitors and parameters determining their effectiveness as pollinators, i.e., vector and stigma pollen load per visit, seed or fruit set per visit, loss of pollen, etc. (Young 1988, Inouye et al. 1994, Gomez and Zamora 1999), for entire communities of plants and their animal visitors. Because of the logistical demands of quantifying pollinator effectiveness, data usually collected on a community level generally represent visitation data or analysis of pollen loads on the presumed vectors (e.g., Mommose et al. 1998). Detailed data on pollinator effectiveness have generally focused on one plant and the vectors that visit it (e.g., Schemske and Horvitz 1984, Nilsson 1988, Galen 1989) and may necessarily represent a biased picture of the specificity of pollinators (Waser et al. 1996). It is also clear from many studies (e.g., Schemske and Horvitz 1989, Herrera 1995) that refinement of current concepts of the evolution and maintenance of pollination syndromes will need to incorporate ecological context, specifically the role of temporal and spatial variation in the role of pollinators (Thompson 1994).

Silene virginica (Fire pink) is a widespread plant of eastern North America, which exhibits a suite of classic floral traits thought to be associated with the hummingbird pollination syndrome (Faegri and Van der Pijl 1979, Baker and Baker 1983, Bertin 1989). These floral traits include red tubular flowers producing copious sucrose-rich nectar ($\sim 40 \mu\text{L}$; unpublished data) that is deeply held, no landing pad, no nectar guides, and no detectable floral odor. The goals of this study are: (1) to test whether *S. virginica* is predominantly pollinated by hummingbirds, as suggested by its floral traits and (2) to quantify both temporal (among year) and spatial (among site) variation of effectiveness of hummingbirds as pollinators. Given that there is only one species of hummingbird in eastern North America, the Ruby-throated Hummingbird, *Archilochus colubris*, we hoped that our study system would represent a simplified model of plant-pollinator interactions and thus allow us to quantify the role of nonspecialist pollinators in what appears a priori to be a highly specialized pollination system.

MATERIALS AND METHODS

Study organism and study site

Silene virginica (Caryophyllaceae) is a short-lived, perennial (Dudash and Fenster 1997) found in eastern North America. The bright red flowers are protandrous and highly outcrossing (M. R. Dudash and C. B. Fenster, unpublished data). It flowers from late May through June at our study sites. During the same period of this study we observed no evidence of pollen limitation of female reproductive success (Dudash and Fenster 1997). Hummingbirds appear to be efficient pollinators, transferring several times the number of pollen grains as ovules per visit (Fenster et al. 1996). In following thousands of flowers in both the greenhouse and field over the last eight years, we have never observed autogamous selfing resulting in fruit set in *S. virginica* (M. R. Dudash and C. B. Fenster, unpublished data).

We conducted this study for five years near Mountain Lake Biological Station (Allegheny Mountains, Giles County, Virginia, USA; elevation ~ 1330 m). From 1991–1993 we quantified the roles of various floral visitors as pollinators in two sites, a woodland site and a meadow site. Plants in the woodland site occurred in the shaded understory of an oak-hickory and formerly chestnut forest, while plants in the meadow site grew without a canopy cover along a power line cut. Approximately 2.5 km separated the two study areas, and the meadow site is ~ 50 m higher in elevation and begins flowering about one week later than the woodland site. We chose these two sites because of their striking difference in canopy cover that might in turn lead to a different visitor fauna. Grazing herbivory increased during our study in the meadow, prohibiting us from continuing the experiment in this site after 1993. The study was continued for two additional years, 1994 and

1995, in the woodland site. We observed the Ruby-throated Hummingbird, *Archilochus colubris*, syrphid flies, and solitary bees to visit *S. virginica* in both sites in all years and in one year, year two of our study (1992), *Bombus* spp., visited *S. virginica* at the meadow site (see Plate 1).

Experimental methodology

To estimate the amount of female reproductive success due to hummingbirds vs. invertebrate pollinators, we randomly chose individuals to be placed into two experimental groups; caged and control. In the cage treatment, poultry wire cages (5×5 cm mesh) were placed around individuals prior to flowering to exclude hummingbirds from visiting but allowed complete access to potential invertebrate pollinators. Contrasting relative fruit and seed set (and total seed set in the woodland site) among caged vs. control individuals should reflect an integrated measure of the role of hummingbirds as pollinators. However, our experimental approach likely provides an upper-bound estimate of the role of nonhummingbird visitors as pollinators since it does not take into account efficiency of pollen removal and deposition and minimization of pollen loss (Inouye et al. 1994), which are more likely to favor the predicted pollinator of a pollination syndrome.

Invertebrate pollinators moved freely through the cages (C. B. Fenster, personal observation). The number of control plants was often greater than the experimentally caged plants because we suspected that variation in fruit production would be greater in the control group. In the woodland site we initially enclosed 20, 25, 26, 20, and 26 plants each year and compared them to 25, 27, 34, 43, and 58 control or open-pollinated plants in the study, respectively. In the meadow site we initially enclosed 15, 25, and 21 plants each year and compared them to 25, 30, and 37, control or open-pollinated plants, respectively. Both cage and control plants were distributed evenly along 100-m transects in each site. Results may be based on fewer individuals owing to flower, fruit, and whole plant herbivory.

Statistical analyses

We conducted an analysis of variance (ANOVA) in both sites (woodland and meadow) for the three years of the study (1991–1993) when both sites were simultaneously studied using the PROC GLM option of SAS (SAS 1996, Release 6.12 TSO-40). We also did an additional ANOVA for only the woodland site for the full five years of the study. The first approach allowed us to examine variation in reproductive success between our two experimental treatments across years and between sites. The second approach allowed us to examine yearly variation between treatments in the woodland site for the entire study. The mixed model analyses incorporated treatment as a fixed effect and year as a random effect. We considered site a fixed effect because of prior expectations that the enclosed understory

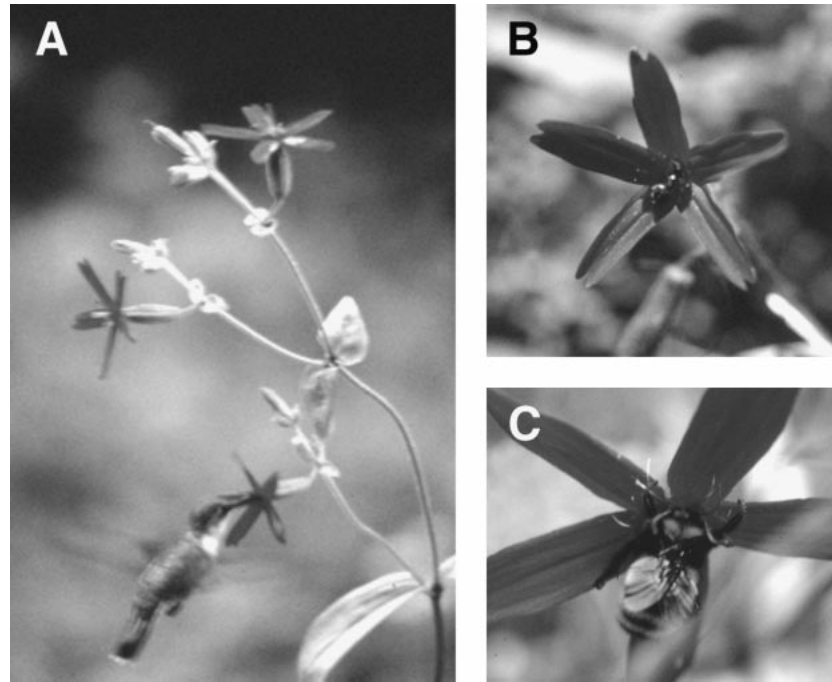


PLATE 1. Common visitors to *Silene virginica* flowers in the Mountain Lake, Virginia, study area: A) hummingbird, B) solitary bee, and C) bumblebee.

habitat of the woodland site may differ in hummingbird visitation rates compared to the relatively exposed habitat of the open meadow site. However, whether site was treated as a fixed or random effect did not influence the outcome of most of the results. The SAS RANDOM statement with the TEST option was used to produce the error mean squares for the mixed-model ANOVA hypotheses tests. This approach requires that a linear combination of mean squares be constructed and *F* tests using a Satterthwaite (1946) approximation are generated. This approximation may generate fractional degrees of freedom in the denominator and/or negative *F* values (pooling the most nonsignificant mean square into the error term usually produces a traditional positive term). ANOVA assumptions were met for all analyses following appropriate transformations.

Dependent variables were percentage fruit set (arcsine square-root transformed), seed set per fruit, given a fruit formed (square-root transformed), and total seed production (square-root transformed). Each trait was analyzed separately. Herbivorized flowers and fruits were removed from analyses of percentage fruit set and number of seed/fruit since we were unable to estimate the number of seed matured. Contrast of total seed production between cage and control plants in the woodland site included plants that experienced some degree of herbivory. Inclusion of these individuals may have slightly biased our results towards observing greater seed production in caged plants, thereby overestimating the roles of invertebrates. Higher rates of herbivory in the meadow site prevented us from con-

trasting total seed production between the two treatments in the meadow site (see *Results: Vegetative and floral characters*). Differences in herbivory levels between the treatments did not effect our estimates of the role of pollinators on percentage fruit set or seed number per fruit, since herbivorized flowers and fruits were not included in these analyses.

To determine whether individuals assigned randomly to our two treatment groups each year differed significantly in total flower production (log transformed), number of ovules per pistil (square-root transformed), and percentage herbivory of flowers and fruits (arcsine square-root transformed) we performed the same series of ANOVA as described above for the reproductive traits.

RESULTS

Vegetative and floral characters

We observed no significant effect of treatment group on total flower production per plant, ovule production per pistil, or percentage herbivory. Plants in both treatment groups produced on average between 2–5 and 4–10 flowers in the woodland and meadow sites, respectively, while ovule number/pistil ranged from 43–68 and 58–62 in the woodland and meadow site, respectively.

Site as a fixed main effect on percentage herbivory was significant ($P < 0.034$). However, when site was assigned as a random main effect, it had a nonsignificant effect on percentage herbivory ($P > 0.125$), after

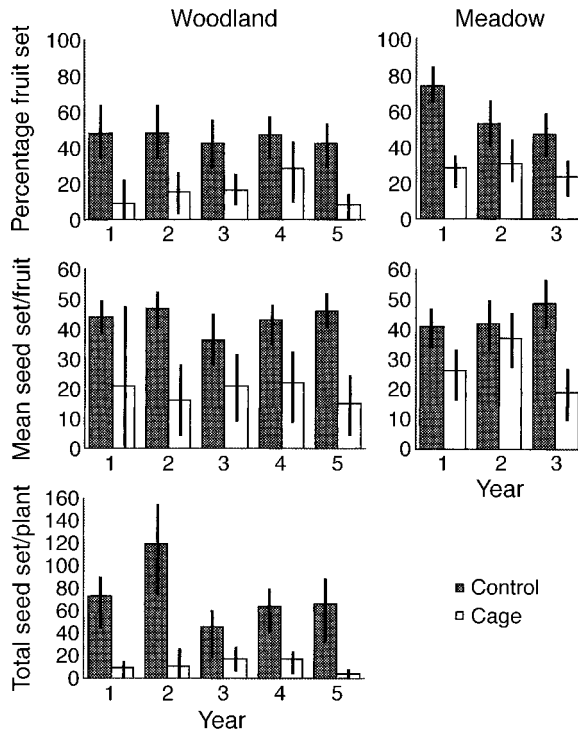


FIG. 1. Female reproductive success of *Silene virginica* accessible to all pollinators (shaded bars) vs. caged treatment plants for which hummingbird access was prohibited (open bars) in two study areas over three years (1991–1993) and over two additional years in the woodland study area. Percentage fruit set, mean seed set per fruit, and total seed production per plant are presented as untransformed means for each treatment group across years. Error bars represent 95% confidence intervals.

pooling site × year and site × treatment effects into the error term to remove negative *F* values for site as a random main effect. When the woodland site was analyzed alone, significant two-way interactions between year and treatment were detected for each trait. The examination of both sites across three years always produced a significant three-way interaction (year × site × treatment) for each trait. One of the most obvious

TABLE 1. Analysis of variance for the dependent variable “arcsine square root of percentage fruit set per plant” of the control and caged treatment groups of *Silene virginica* during 1991–1993 in the woodland and meadow sites.

Source of variation	df	MS	<i>F</i>	<i>P</i>
Year	2	0.25	0.37	0.721
Site	1	3.19	7.45	0.111
Treatment	1	13.98	34.58	0.027
Year × Site	2	0.43	2.66	0.072
Year × Treatment	2	0.41	2.52	0.082
Error	280	0.16		

Notes: The Site × Treatment and Year × Site × Treatment interactions were most nonsignificant and pooled into the error term to remove a negative *F* value. Mean squares are based on Type III sums of squares.

TABLE 2. Analysis of variance for the dependent variable “arcsine square-root of percentage of fruit set per plant” of the control and caged treatment groups of *Silene virginica* during 1991–1995 in the woodland site.

Source of variation	df	MS	<i>F</i>	<i>P</i>
Year	4	0.47	1.87	0.279
Treatment	1	13.38	53.10	0.002
Year × Treatment	4	0.25	1.16	0.330
Error	276	0.22		

Note: Mean squares are based on Type III sums of squares.

causes of these interactions was the greater reduction in herbivory in the caged vs. control plants in the meadow site, vs. the woodland site owing to the cage protection from grazing mammals (i.e., deer, woodchuck). Mean percentage herbivory of flowers and fruit per plant in the meadow site ranged from 16–23% for control plants and 3–8% for caged plants across the three years of the study. Herbivory levels were generally lower in the woodland site, ranging from 1–11% for control plants and 1–8% for caged plants for the five years of the study and were not significantly different from one another.

Female reproductive success

Despite lower herbivory, the caged treatment group suffered a significant reduction in fruit production compared to the open-pollinated control group throughout the study in both sites (Fig. 1; woodland and meadow: Table 1; woodland alone: Table 2). All interactions in both analyses were nonsignificant, and when the two least significant (site × treatment and year × site × treatment) were pooled into the error term all *F* values were positive. Upon fruit maturation, the average number of seed produced per fruit was also significantly less in the experimental cages compared to the open-pollinated control group (Fig. 1; woodland and meadow: Table 3; woodland alone: Table 4). The full model for both sites (Model = year/treatment/site) again produced a negative *F* value, which required the pooling of each highly nonsignificant interaction into the error term (year × site, year × treatment, and site × treatment). After pooling, the only significant main effect on seeds/fruit was still the cage vs. no cage treatment

TABLE 3. Analysis of variance for the dependent variable “square root of total seed set per fruit per plant” of the control and caged treatment groups of *Silene virginica* during 1991–1993 in the woodland and meadow sites.

Source of variation	df	MS	<i>F</i>	<i>P</i>
Year	2	4.12	0.61	0.567
Site	1	4.10	0.64	0.445
Treatment	1	145.16	22.73	0.001
Year × Site × Treatment	7	7.76	3.47	0.002
Error	206	2.23		

Notes: All highly nonsignificant two-way interactions were pooled into the error term to remove negative *F* values. Mean squares are based on Type III sums of squares.

TABLE 4. Analysis of variance for the dependent variable "square root of total seed set per fruit per plant" of the control and caged treatment groups of *Silene virginica* during 1991–1995 in the woodland site.

Source of variation	df	MS	F	P
Year	4	1.12	0.55	0.712
Treatment	1	144.54	71.42	0.0002
Year × Treatment	4	2.04	1.04	0.388
Error	166	1.96		

Note: Mean squares are based on Type III sums of squares.

effect ($P < 0.001$), and the three-way year × site × treatment interaction was significant ($P < 0.002$). The woodland site when examined alone also exhibited a significant reduction in seed set per fruit ($P < 0.0002$) of the caged group compared to the open-pollinated control group while the interaction was nonsignificant. Pooling nonsignificant interaction terms into the error term had no influence on the overall main effects except to remove negative F values with the full model.

In the woodland site we observed a significant reduction in overall seed production per plant in the caged treatment group compared to the open-pollinated treatment group (Fig. 1; Table 5). We detected no significant main effect of year on overall seed production but did detect a significant year × treatment effect ($P < 0.025$).

To examine the relative importance of hummingbirds as pollinators of *S. virginica* throughout this study we constructed a ratio of relative reproductive success for the three reproductive traits that factors out the contribution of invertebrate pollinators to female reproductive success of the control plants: [(control – cage)/control] (Table 6). The ratio should be considered a measure of the dependence of *S. virginica* on hummingbird pollination. Based on this ratio, across the five years of the study and two sites, hummingbirds are responsible for at least 40–81% of the fruit set, 12–67% of the amount of seed set per fruit, and in the woodland site, 62–95% of the total seed of the control plants.

DISCUSSION

Hummingbirds are the most important pollinator of *S. virginica* in our study sites, but their importance varies across years and between sites (Fig. 1 and Table 6). In the one analysis where interaction effects on female reproductive success were detected, it was a three-way interaction among site, year, and treatment on mean seed set per fruit (Table 3). To understand this interaction more fully we conducted one-way ANOVAs for each year and site (analyses not shown). After a sequential Bonferroni correction (Rice 1989) we found that the control plants had significantly higher seed set per fruit than the caged plants in all years but year two (1992) in the meadow. In year two, visits by the invertebrates could have accounted for nearly all seed set per fruit in the meadow site. The higher seed set

TABLE 5. Analysis of variance for the dependent variable "square root of total seed production per plant" of the control and caged treatment groups of *Silene virginica* during 1991–1995 in the woodland site.

Source of variation	df	MS	F	P
Year	4	44.71	0.97	0.513
Treatment	1	1655.56	33.89	0.004
Year × Treatment	4	49.34	2.84	0.025
Error	276	17.37		

Note: Mean squares are based on Type III sums of squares.

per fruit of the caged plants in the meadow in year two may reflect the presence of *Bombus* spp. visitors, the only year they appeared in our study. The rare appearance of *Bombus* spp. as pollinators of *S. virginica* mirrors observations of pollination of *Ipomopsis aggregata* (Pleasants and Waser 1985; N. M. Waser, *personal communication*). Roughly every 10–15 yr, when hummingbirds are relatively scarce, standing crop levels of nectar are high enough in the corolla tube to be accessible to *Bombus* spp. Normally, the presence of hummingbirds may preempt *Bombus* spp. visitation of *Ipomopsis* because of direct interactions between the two taxa or through reduction of *Bombus* spp. foraging efficiency because the standing crop of nectar is depressed (Pleasants and Waser 1985). Overall, our observations and those of N. Waser and colleagues suggest that morphological features alone frequently do not act as barriers to pollination by "inappropriate" pollinators. Flowers represent a resource to visitors and plants are constrained in their ability to restrict access to those resources. Therefore we might expect a priori that specialized pollination systems will reflect the evolution of rewards and how they are presented rather than the evolution of attractive features, e.g., specialized pollination of orchids by male euglossine bees for fragrances (Dodson et al. 1969) or pollination of *Daléchampia* by resin collecting bees (Armbruster 1992,

TABLE 6. Dependence of *Silene virginica* reproductive success on hummingbird visitation.

Year	Mean fruit set per plant		Mean seed set/fruit per plant		Total seed set per plant
	Woodland	Meadow	Woodland	Meadow	Woodland
1	0.81	0.62	0.52	0.37	0.89
2	0.69	0.42	0.66	0.12	0.92
3	0.62	0.51	0.42	0.61	0.62
4	0.40		0.49		0.75
5	0.81		0.67		0.95

Notes: Values expressed are the ratio of [(control – cage)/control] untransformed means for each year and site. The ratio indicates the proportion of reproductive success solely attributable to hummingbird visitation. Mean fruit set represents the percentage of successful fruit formed per flower, mean seed set represents the average seed produced, given a fruit matured, and total seed set represents the total seed produced per plant including those flowers that produced no successful fruit.

1993). Similarly, storing copious nectar rewards at the base of long pendulant corollas may serve to prevent all but long-tongued pollinators from having access to the reward of hummingbird pollinated flowers.

Our documentation of year and site effects on the consequences of restricting the types of pollinators to *S. virginica* corresponds to other studies in which important temporal and spatial variation in the composition of pollinators visiting particular plant species has been observed (Bertin 1982, Schemske and Horvitz 1984, Herrera 1988, 1995, Horvitz and Schemske 1990, Pettersson 1991, Thompson and Pellmyr 1992, Fishbein and Venable 1996). In sum, these results suggest that the role pollinators play as selective agents may be context or community dependent on the composition of the pollinator community found in a given population of the focal plant species (Thompson 1994). If pollination syndromes arise and are maintained by similar selection pressures, then we can expect that the intensity and perhaps even the direction of selection will vary temporally and spatially. Indeed, varying selection pressures appears to be a hallmark of studies quantifying selection on floral traits (Campbell 1989, Schemske and Horvitz 1989, Johnston 1991, Fenster and Ritland 1994, O'Connell and Johnston 1998).

The data presented here illustrates the importance of documenting the contribution of pollinators to total seed production per plant whenever possible, in addition to its components (fruit set and seed set/fruit). Correlation analyses (not shown) between percentage fruit set and seed set per fruit for individuals were conducted for each treatment, year, and site combination and generally demonstrated no association between the probability to form a fruit and the number of seed matured in a fruit on a per plant basis. Our data suggest that the role of invertebrate pollinators may be inflated based on the individual fitness components. Total seed set per plant more clearly demonstrates that hummingbirds are the dominant pollinator while invertebrates appear to play a more secondary but still potentially important role as pollinators in this system. Our measure of the roles of the different invertebrates as pollinators may be upwardly biased since we only quantified the role of nonhummingbird visitors in the absence of hummingbirds. Hummingbirds, because of higher efficiency of pollen transfer, etc., may be responsible for most of the pollination of *S. virginica* in the presence of the invertebrate pollinators. Thus our measure of the relative importance of hummingbird pollinations (Table 6) is more properly a metric of the necessity of hummingbird pollination or dependence on hummingbird visitation for full reproductive success.

Other studies have also documented a concordance between floral traits and the expected pollinator based on those traits, e.g., hummingbirds account for most of the pollination of the red-flowered trumpet creeper (Bertin 1982). However, Fishbein and Venable (1996)

documented that *Bombus* and *Apis* (Hymenoptera) are the most important pollinators of the milkweed, *Asclepias tuberosa*, where floral traits would have predicted Lepidopterans as the primary pollinators. Their results stress that identity of effective pollinators must be based on quantitative studies conducted over several years. Mixed pollination by hummingbirds, bees, and flies has also been documented in other studies. Waser (1979), using similar approaches as used here, found that carpenter bees are responsible for upwards of 50% of seed in ocotillo plants which also exhibit floral traits normally associated with hummingbird pollination. *Penstemon pseudospectabilis* displays a mixture of floral traits associated with both insect and hummingbird pollination (Lange and Scott 1999) and has a mixture of hummingbirds, and small and large bees accounting for seed set (Reid et al. 1988, Lange and Scott 1999). This supports the notion that both types of pollinators are selective agents for floral features in this species. The general conclusion that can be drawn from our work and those of others is that pollination is rarely achieved by one guild of pollinator, even when plants exhibit floral traits that have been historically associated with pollination syndromes. The unanswered question is: What role then do these "minor" pollinators play in the evolution of floral traits?

The role of invertebrate pollinators of *S. virginica* suggests that in the absence of hummingbirds they could act as selective agents favoring the disruption of floral characters associated with pollinator specificity to hummingbirds. Specialization in pollination syndromes is not a universal evolutionary trend. Derived floral traits are sometimes more generalized while ancestral traits are often specialized (McDade 1992, Armbruster and Baldwin 1998). A variety of visitors suggests that selective agents for diversification are ever present since each has the potential to be a dominant pollinator if the composition of pollinators should change (Baker 1963).

Since hummingbirds are important pollinators of *S. virginica* and *S. virginica* exhibits many floral traits associated with the hummingbird pollination syndrome, it is likely that hummingbirds are the most important selective agent responsible for the evolution and maintenance of these traits. Although we have quantified a larger role of hummingbirds as pollinators of *S. virginica* compared to the invertebrate pollinators, our data presented here do not allow us to directly quantify the role of any of the visitors as selective agents. To quantify the adaptive significance of floral traits, especially as to whether they reflect a pollination syndrome, it will be necessary to establish the differential ability of pollinators to act as selective agents on floral characters (Wilson and Thomson 1996). Even though particular visitors may be important pollinators, they may pollinate flowers indiscriminately with regards to variation of floral traits found in populations. These "indiscriminate" pollinators would not exert any

selective pressures on floral traits while those that do pollinate discriminately will exert selective pressures and may be responsible for the evolution and maintenance of pollination syndromes. For example, Schemske and Horvitz (1984, 1989) document that the most effective pollinators of *Calathea ovandensis*, an understory neotropical herb, are two relatively short-tongued Hymenoptera, *Rhathymus* sp. and *Bombus medius*. Both of these species were effectively absent in two of the three years of their study. In the one year that they were present they constituted only 9% of all visitors. However, only in this year were Schemske and Horvitz able to detect significant selection intensities such that plants producing flowers better adapted to pollination by short-tongued pollinators experienced relatively greater reproductive success. Therefore, quantifying pollinator importance in terms of pollen receipt and export may still lead to the misidentification of important selective agents leading to or contributing to the maintenance of pollination syndromes. There are a diverse array of approaches available to quantify selection on floral features, including phenotypic selection analysis (Lande and Arnold 1983, Schemske and Horvitz 1989), experimental manipulation of traits (Peakall and Handel 1993), as well as comparative approaches (Fenster 1991, Armbruster 1996, Herrera 1996). In particular, to prove the utility of the concept of the pollination syndrome, studies are needed that explicitly demonstrate that pollinators associated with a particular syndrome actually select for traits associated with that syndrome (Armbruster et al. 2000).

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LITERATURE CITED

- Armbruster, W. S. 1992. Phylogeny and the evolution of plant-animal interactions. *BioScience* **42**:12-20.
- Armbruster, W. S. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* **45**:1480-1505.
- Armbruster, W. S. 1996. Evolution of floral morphology and function: an integrative approach to adaptation, constraint, and compromise in *Dalechampia* (Euphorbiaceae). Pages 241-272 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York, New York, USA.
- Armbruster, W. S., and B. G. Baldwin. 1998. Switch from specialized to generalized pollination. *Nature* **394**:632.
- Armbruster, W. S., C. B. Fenster, and M. R. Dudash. 2000. Pollination "principles" revisited: specialization, pollination syndromes, and the evolution of flowers. Norwegian Academy of Sciences, *in press*.
- Baker, H. G. 1963. Evolutionary mechanisms in pollination biology. *Science* **139**:877-883.
- Baker, H. G., and I. Baker. 1983. Floral nectar sugar constituents in relation to pollinator type. Pages 117-141 in C. E. Jones and R. J. Little, editors. *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, New York, USA.
- Bertin, R. I. 1982. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). *American Journal of Botany* **69**:122-134.
- Bertin, R. I. 1989. Pollination biology. Pages 23-83 in W. G. Abrahamson, editor. *Plant-animal interactions*. McGraw-Hill, New York, New York, USA.
- Bessey, C. E. 1915. The phylogenetic taxonomy of flowering plants. *Annals of the Missouri Botanical Garden* **2**:108-164.
- Bradshaw, H. D., Jr., S. M. Wilbert, K. G. Otto, and D. W. Schemske. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkey flowers (*Mimulus*). *Nature* **376**:762-765.
- Campbell, D. R. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. *Evolution* **43**:318-334.
- Darwin, C. 1859. *On the origin of species by means of natural selection*. Murray, London, UK.
- Darwin, C. 1862. *On the various contrivances by which British and foreign orchids are fertilized*. Murray, London, UK.
- Dodson, C. H., R. L. Dressler, H. G. Hills, R. M. Adams, and N. H. Williams. 1969. Biologically active compounds in orchid fragrances. *Science* **164**:1243-1249.
- Dudash, M. R., and C. B. Fenster. 1997. Multiyear study of pollen limitation and cost of reproduction in iteroparous *Silene virginica*. *Ecology* **78**:484-493.
- Eriksson, O., and B. Bremer. 1992. Pollination systems, dispersal modes, life forms and diversification rates in angiosperm families. *Evolution* **46**:258-266.
- Faegri, K., and L. van der Pijl. 1979. *The principles of pollination ecology*. Pergamon, Oxford, UK.
- Fenster, C. B. 1991. Selection on floral morphology by hummingbirds. *Biotropica* **23**:98-101.
- Fenster, C. B., C. B. Hassler, and M. R. Dudash. 1996. Fluorescent powder is a good analog of pollen in *Silene virginica* (Caryophyllaceae). *Canadian Journal of Botany* **74**:189-193.
- Fenster, C. B., and K. Ritland. 1994. Evidence for natural selection on mating system in *Mimulus* (Scrophulariaceae). *International Journal of Plant Sciences* **155**:588-596.
- Fishbein, M., and D. L. Venable. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* **77**:1061-1073.
- Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumble bees and the alpine skypilot, *Polemonium viscosum*. *Evolution* **43**:882-890.
- Gomez, J. M., and R. Zamora. 1999. Generalization vs. specialization in the pollination system of *Hormathophylla spinosa* (Cruciferae). *Ecology* **80**:796-805.
- Grant, V., and K. A. Grant. 1965. *Flower pollination in the phlox family*. Columbia University Press, New York, New York, USA.
- Herrera, C. M. 1988. Variation in mutualisms: the spatiotemporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* **35**:95-125.
- Herrera, C. M. 1995. Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* **76**:1516-1524.
- Herrera, C. M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. Pages 65-87 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York, New York, USA.

- Hodges, S. A., and M. L. Arnold. 1994. Columbines: a geographically widespread species flock. *Proceedings of the National Academy of Sciences (USA)* **91**:5129–5132.
- Horvitz, C. C., and D. W. Schemske. 1990. Spatiotemporal variation in insect mutualists of a neotropical herb. *Ecology* **71**:1085–1097.
- Inouye, D. W., D. E. Gill, M. R. Dudash, and C. B. Fenster. 1994. A model and lexicon for pollen fate. *American Journal of Botany* **81**:1517–1530.
- Johnston, M. O. 1991. Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* **45**:1468–1479.
- Knuth, P. 1908. *Handbook of flower pollination. Volume II. Observations on flower pollination made in Europe and the Arctic regions on species belonging to the natural orders Ranunculaceae to Stylidiaceae.* Clarendon, Oxford, UK.
- Knuth, P. 1909. *Handbook of flower pollination. Volume II. Observations on flower pollination made in Europe and the Arctic regions on species belonging to the natural orders Goodenovieae to Cycadeae.* Clarendon, Oxford, UK.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* **37**:1210–1226.
- Lange, R. S., and P. E. Scott. 1999. Hummingbird and bee pollination of *Penstemon pseudospectabilis*. *Journal of the Torrey Botanical Society* **126**:99–106.
- McDade, L. A. 1992. Pollinator relationships, biogeography, and phylogenetics. *BioScience*, **42**:21–26.
- Momose, K., T. Yumoto, T. Nagamitsu, M. Kato, T. Nagamitsu, S. Sakai, R. D. Harrison, T. Itioka, A. A. Hamid, and T. Inoue. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. *American Journal of Botany* **85**:1477–1501.
- Muller, N. H. 1883. *The fertilization of flowers.* Translated by D. Thompson. Macmillan, London, UK.
- Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. *Nature* **334**:147–149.
- O'Connell, L. M., and M. O. Johnston. 1998. Male and female pollination success in a deceptive orchid, a selection study. *Ecology* **79**:1246–1260.
- Ollerton, J. 1996. Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. *Journal of Ecology* **84**:767–769.
- Ollerton, J. 1998. Sunbird surprise for syndromes. *Nature* **84**:726–727.
- Peakall, R., and S. N. Handel. 1993. Pollinators discriminate among floral heights of a sexually deceptive orchid: implications for selection. *Evolution* **47**:1681–1687.
- Pettersson, M. W. 1991. Pollination by a guild of fluctuating moth populations: options for unspecialization in *Silene vulgaris*. *Journal of Ecology* **79**:581–604.
- Pleasants, J. M., and N. M. Waser. 1985. Bumblebee foraging at a "hummingbird" flower: reward economics and floral choice. *The American Midland Naturalist* **114**:283–291.
- Reid, W. H., P. Sensiba, and C. E. Freeman. 1988. A mixed pollination system in *Penstemon pseudospectabilis* M. E. Jones. *Great Basin Naturalist* **48**:489–494.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**:223–225.
- Robertson, C. 1928. *Flowers and insects: lists of visitors of four hundred and fifty-three flowers.* Charles Robertson, Carlinville, Illinois, USA.
- SAS. 1996. Release 6.12 TSO-40. SAS Institute, Cary, North Carolina, USA.
- Satterthwaite, F. E. 1946. An approximate distribution of estimates of variance components. *Biometrics Bulletin* **2**:110–114.
- Schemske, D. W., and C. C. Horvitz. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* **225**:519–521.
- Schemske, D. W., and C. C. Horvitz. 1989. Temporal variation in selection on a floral character. *Evolution* **43**:461–465.
- Sprengel, C. K. 1793. *Discovery of the secret nature in the structure and fertilization of flowers.* [Translated from German in 1996 by Peter Haase.] Pages 3–43 in D. G. Lloyd and S. C. H. Barrett, editors. 1996. *Floral biology: studies on floral evolution in animal-pollinated plants.* Chapman and Hall, New York, New York, USA.
- Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review Ecology and Systematics* **1**:307–326.
- Stebbins, G. L. 1974. *Flowering plants. Evolution above the species level.* Harvard University Press, Cambridge, Massachusetts, USA.
- Thompson, J. N. 1994. *The coevolutionary process.* The University of Chicago Press, Chicago, Illinois, USA.
- Thompson, J. N., and O. Pellmyr. 1992. Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology* **73**:1780–1791.
- Waser, N. M. 1979. Pollinator availability as a determinant of flowering time in *Ocotillo* (*Fouquieria splendens*). *Oecologia* **39**:107–121.
- Waser, N. M. 1983. The adaptive nature of floral traits: ideas and evidence. Pages 241–285 in L. A. Real, editor. *Pollination biology.* Academic Press, New York, New York, USA.
- Waser, N. M., and L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* **77**:1043–1060.
- Wilson, P., and J. D. Thomson. 1996. How do flowers diverge? Pages 88–111 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology: studies on floral evolution in animal-pollinated plants.* Chapman and Hall, New York, New York, USA.
- Young, H. J. 1988. Differential importance of beetle species pollinating *Diffenbachia longispatha* (Araceae). *Ecology* **69**:832–844.