

TEMPORAL AND SPATIAL VARIATION IN POLLINATION OF A MONTANE HERB: A SEVEN-YEAR STUDY

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Abstract. Pollination by animals is critical to sexual reproduction of most angiosperms. However, little is known about variation in pollination service to single plant species. We report results of a long-term study of *Ipomopsis aggregata*, a semelparous montane herb whose flowers are visited by hummingbird and insect pollinators as well as “floral larcenists.” We censused flower visitors over seven summers at permanent study sites separated by several hundred meters, and counted pollen delivered to flowers on a subset of plants observed for visitation. The species composition of the community of visitors varied significantly across years and within the flowering season; sites varied significantly only in the magnitude of parallel annual changes in the visitor community. Rates of flower visitation fluctuated over an order of magnitude or more. Variation in mean stigma pollen load among plants flowering in the same site and year was explained by a causal path model in which visitation rates by pollinators and larcenists had linear positive and negative effects, respectively. A simplified model including only pollinators explained almost as much variance as did the full model. However, qualitatively different parameter estimates were produced by an analogous causal model based on population means across site–year combinations. Discrepant results from within- and between-population levels of analysis suggest that pollen receipt is influenced by environmental factors that vary among sites and years, as well as by pollinator visit rates. We present a heuristic causal model that includes such factors, and we note its implications for ecological and evolutionary studies of pollination.

Key words: field study; floral larcenists; floral visitation rates; flower-visitor community; hummingbirds; insects; *Ipomopsis aggregata*; long-term study; path analysis; plant population dynamics; pollen delivery; pollination services; structural equation modeling.

INTRODUCTION

Pollination of flowering plants by animals is a ubiquitous interaction in terrestrial ecosystems (Nabhan and Buchmann 1997), comprising a critical ecosystem service (Costanza et al. 1997) that is increasingly threatened by human activity (Kearns et al. 1998). The scientific study of pollination dates back almost 250 years (Baker 1983), reflecting the conspicuous nature of this interaction and its central ecological role. This long history of study has revealed much about differences among plant species in major pollinators, and among pollinator species in floral affinities. In contrast, relatively little attention has been given to variation over time and space in visitor faunas or pollination service (exceptions include Herrera 1988, Horvitz and Schem-

ske 1990, Pettersson 1991, Fishbein and Venable 1995, Fenster and Dudash 2001).

Surely it made sense for ecologists to begin by studying the “average” or “usual” state of pollination interactions. And perhaps the characterization of variance has seemed tedious and uninteresting in itself. But because visitation to flowers and movement of pollen is one (although not the only) determinant of seed production, and seed production is one (although not the only) determinant of subsequent demography (Crawley 1990, Parker 1997, Turnbull et al. 2000), variation in pollination service has the potential to profoundly influence the ecological dynamics of plant populations and communities (Ashman et al. 2004).

As part of a long-term study of the links between pollination, seed production, and plant population dynamics, we observed flower visitation to *Ipomopsis aggregata* (Polemoniaceae) over seven years at three study sites, and for single years at two additional sites. Our goal was to characterize temporal and spatial variation in rates of flower visitation and in composition of the visitor fauna, which includes not only pollinating birds and insects but also nectar robbers and pollen

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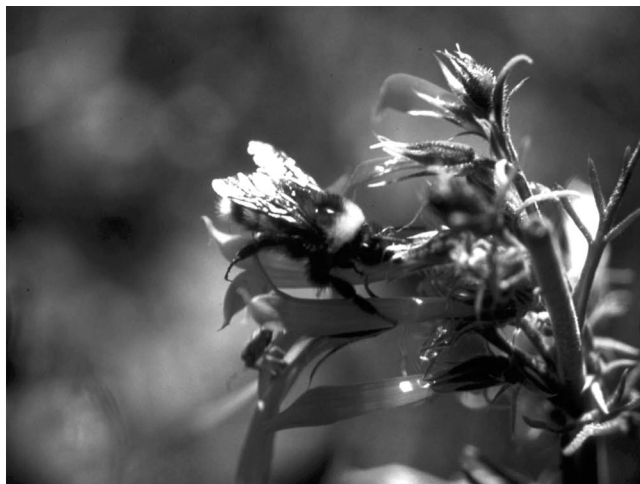


PLATE 1. (Left) A queen bumble bee, *Bombus appositus*, collecting nectar from scarlet gilia, *Ipomopsis aggregata*, at the Rocky Mountain Biological Laboratory. Scarlet gilia is pollinated by hummingbirds and by these long-tongued bees and other insects, whose services vary in time and space. Photo credit: David W. Inouye. (Right) *Bombus occidentalis* nectar-robbing a flower of *Ipomopsis aggregata*. Photo credit: Brad W. Taylor.

thieves (“larcenists” sensu Irwin et al. 2001). To relate variation in floral visitation to pollination, in three years we also scored pollen loads on stigmas of flowers. This information allowed us to address whether, and how, variation in visitation to flowers translates into variation in pollen receipt. A positive relationship between visitation and pollen receipt (or donation) is assumed by many studies of natural selection on floral traits, and is also a prerequisite, along with pollen limitation of seed set, for pollination to have a demographic effect. Nonetheless, the strength and shape of the relationship, much less its variation in natural populations, have rarely been quantified.

METHODS

Study organism and study sites

Ipomopsis aggregata ssp. *aggregata* (Pursh) V. Grant is a self-incompatible, perennial, semelparous herb of montane habitats throughout the western United States (Grant and Wilken 1986; see Plate 1). Around the Rocky Mountain Biological Laboratory in western Colorado (38°58' N, 106°59' W; elevation 2900 m), it is common in dry meadows and disturbed sites. Plants establish solely from seeds that lack extended dormancy. Flowering stalks produce from a few to several hundred red, tubular flowers in a paniculate raceme. The protandrous flowers continuously secrete dilute (~25% mass/volume) nectar, producing 3–6 μ L over 24 h (Pleasants 1983). Variation in several floral characters influences pollen removal and deposition (Campbell et al. 1994, 1996), and seed set can be pollen limited (Hainsworth et al. 1985, Campbell 1991, Campbell and Halama 1993).

Observations and calculations of visitation rates

We observed visitation to focal plants in six representative meadows separated by 150–575 m. Three meadows (P, T, and V) were observed from 1996 through 2002; one (CC) in 1996 only; and two others (OH and SN) in 1997 only. In each summer, observations began when ~30% of plants had started to flower at each site, and continued for 6 wk, except in 1997 (5 wk), and in 1999 and 2000 (8 wk). Peak flowering occurred between weeks 2 and 4. Each site was observed each week for three periods of equal length, one in the morning (between 07:00 and 11:00 h), one at midday (11:00–14:00 h), and one in the afternoon (14:00–18:00 h), except that observations at OH and SN were not blocked by week, total observation time at these sites varied among weeks, and the ratio of morning:midday:afternoon observation time was approximately 4:2:1. We did not conduct nocturnal observations because previous investigation (E. J. Meléndez-Ackerman, *personal communication*) had detected very little nocturnal visitation.

In 1996 we observed all plants within 5–6 focal plots at each site. Plots measured 2 \times 2 m and were chosen to span the range of flowering-stalk density at each site (<1 to >3.8 stalks/m²). In subsequent years we observed 12–31 focal plants at each site, choosing individuals haphazardly with respect to size. After flowering stalks began to bolt, but before first flowers opened, focal plants were identified with numbered cards placed far enough away that they did not interfere with pollination. For each observation period, a single observer recorded number of open flowers for each focal plant or plot, and then watched all plants or plots simultaneously. Time of day, visitor identity, plant or

plot identity, and number of flowers probed were recorded for each plant or plot visited. Visitation rates were low enough that simultaneous visits by multiple individuals, which might cause some visits to be missed, were rare. Reliably distinguished visitor groups were hummingbirds (broad-tailed, *Selasphorus platycercus*, rufous, *S. rufus*, and rare calliope, *Stellula calliope*), long-tongued bumble bees (queens of *Bombus appositus* and *B. flavifrons* foraging legitimately for nectar), nectar-robbing bumble bees (*Bombus occidentalis*), butterflies (*Papilio rutulus* and *P. gothica*), hawkmoths (*Hyles lineata*), wasps (vespoid and sphecoïd) and small insects (syrphid and muscoïd flies, and solitary bees). Our observation methods did not reliably census ants and small pollen-eating beetles; these visitors were therefore excluded from analysis. Although some solitary bees collect nectar by crawling into the floral tube and may pollinate flowers (Waser 1978), most wasps and small insect visitors eat or collect pollen but do not enter the floral tube and probably do not pollinate.

We expressed visitation rate as the number of probes by a given type of visitor per open flower per hour of observation, calculated by summing the total flower visits to a plant (or plot) over the time period of interest (either a week's block of observations, or the entire season; see *Analysis of . . .* subsections, below), and dividing by the sum of flower-hours of observation over the same period. This method weights equally each flower observed for an hour, whether observed during morning, midday, or afternoon, or during start, peak, or end of the flowering season. We did not average over observation periods to avoid giving equal weight to data from periods of low and high flower density.

Stigma pollen loads

Beginning in the second week of flowering, we collected stigmas weekly from three plants per plot in 1996, and from each focal plant in 1997 and 1998. Stigmas were collected only if they had wilted, an indication that at least 24 h had passed since the corolla had abscised, by which time ovules have been fertilized (Waser and Price 1991). All stigmas in the appropriate stage were collected from each plant sampled, to a maximum of 5 stigmas/wk. We mounted stigmas on microscope slides, stained with basic fuchsin gel, and counted pollen grains with a compound microscope. Counts included self-pollen, which does not effect seed set, because we chose not to emasculate flowers. Emasculation influences natural visitation behavior as well as the mechanics of pollen deposition (Price and Waser 1982, Harder 1990). Stigma pollen loads collected from each focal plant or plot were averaged to obtain a total-season average pollen load.

Analysis of spatial and temporal variation in visitation

We used MANOVA and canonical discriminant analysis (GLM and CANDISC procedures of SAS; SAS

Institute 1997) to characterize the relative magnitude of variation in visitation, in space (among sites) and in time (among years and among periods within flowering seasons), and to evaluate the types of visitors that contribute most to the variation observed. Visitors were grouped into five types: hummingbirds, long-tongued bumble bees, Lepidoptera (hawkmoths + butterflies), nectar-robbing bumble bees, and pollen-eating insects (wasps + flies + solitary bees). For analyses of among-site and among-year variation, we used data from the three sites for which we had seven years of data. Year and site were treated as random effects and tested over the site \times year interaction; site \times year interaction was tested over variation among plants or plots. To assess within-season variation, we used data from all sites and years with at least five weeks of observation. We calculated visitation rates by week for each site and year, then took residuals from the simple across-week mean for each site and year. We divided the season into three periods represented in all seven years—"Early" (weeks 1–2), "Middle" (weeks 3–4, which generally included peak flowering), and "Late" (weeks 5–8), and used MANOVA and canonical discriminant analysis to look for differences among these parts of the season in patterns of residuals.

Analysis of the relationship between visitation and pollen deposition

We used structural-equation modeling (Mitchell 1993, Tabachnick and Fidell 1996, Shipley 2000) to explore how variation in observed visitation rates translates into variation in pollen deposition on stigmas. The relationship between visitation rates and pollen deposition was evaluated on two scales: within sites, i.e., among focal plots or plants observed simultaneously within single sites during single summer flowering seasons (hereafter "within-site" level); and among means for each site and flowering-season (hereafter "site-year" level). For the former analysis we used data from four sites (CC, P, V, T) and three years (1996, 1997, 1998) for which we had matched data on visitation and stigma pollen loads from a total of 101 focal units (23 plots in 1996, 60 plants in 1997, and 18 plants in 1998). This within-site analysis used visitation and pollen data centered on site-year means to remove the contribution of variation across sites and years to the covariance structure. In contrast, analysis of variation at the site-year level used overall means across focal units from the 12 site-year combinations (four sites in 1996, five in 1997, three in 1998) for which we had both pollen and visitation data.

The full model that we propose (Fig. 1) postulates that rates of visitation by hummingbirds, Lepidoptera, and long-tongued bumble bees have positive direct effects on stigma pollen loads, because these visitors are pollinators (i.e., they contact anthers and stigmas while feeding from flowers and do not eat or systematically collect pollen; Waser 1978). The model further pos-

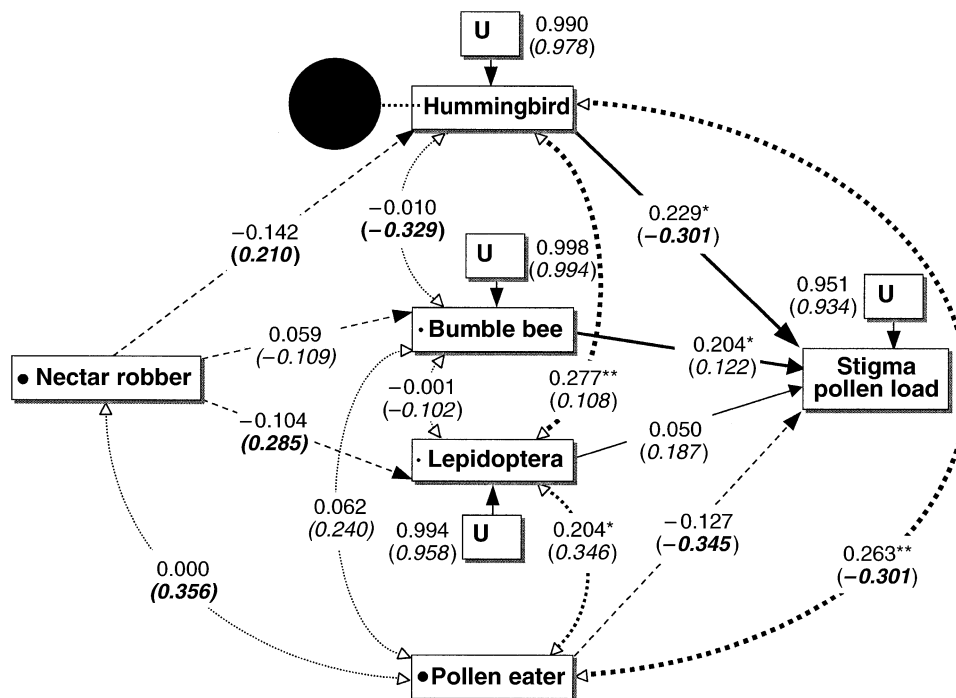


FIG. 1. Causal model relating visits from five classes of visitors to variation in stigma pollen loads. Diameters of the solid circles inside or beside visitor boxes indicate the relative proportion of total visits contributed by each class of visitor. Lines with single-direction solid arrows indicate a causal path; solid lines indicate positive effects, and dashed lines negative effects. Bi-directional lines with two open arrow heads and with dotted lines indicate unexplored correlations among variables. “U” indicates unexplained variation $[(1 - R^2)^{0.5}]$ associated with dependent variables. Line thickness is proportional to the significance of the within-population path coefficient (* $P < 0.05$; ** $P < 0.01$), which is the upper number of each pair of numbers. The lower number (in parentheses) is the corresponding path coefficient from site-year analysis. Boldface type indicates that the site-year coefficients are outside the 95% confidence interval for within-population analysis.

tulates that pollen-eating insects have a direct negative effect on pollen loads, because their consumption or collection of pollen from anthers reduces the pollen carried by pollinators, and hence the amounts deposited on stigmas during each visit. Nectar robbers rarely contact stigmas, and they reduce visitation by hummingbirds (Irwin and Brody 1998); thus the model postulates only an indirect effect on stigma pollen loads mediated through depression of visitation rates by pollinators. Finally, we included unanalyzed correlations among pollinators, between nectar robbers and pollen eaters, and between pollen eaters and nectar robbers. To see whether the common practice of ignoring non-pollinating visitors in pollination studies is warranted, we also compared the fit of the model in Fig. 1 with a simplified one without nectar robbers or pollen eaters. Of necessity this was a qualitative comparison because the two models are not nested, and the simplified model is just-identified (i.e., there are no degrees of freedom available to test the significance of the model) (Tabachnick and Fidell 1996).

We used PROC CALIS in SAS (METHOD = ML) (SAS Institute 1997) to estimate parameters of the model. Following Tabachnick and Fidell (1996), a chi-square test was used first to assess whether an “in-

dependence model,” which assumes no relationships among the variables (i.e., all covariances equal zero), provided a good fit to observed patterns of covariance. If the fit was poor (significant chi-square), then the overall fit between observed patterns of covariance and those estimated from the hypothesized model was evaluated; a significant drop in chi-square of the hypothesized model relative to the independence model, and nonsignificant model chi-square, indicate a good fit. Wald and Lagrange multiplier tests were used to explore whether model fit could be improved by removing individual estimated parameters from the model by fixing them to zero, or by estimating parameters that had been fixed to zero, respectively.

Sample size for the within-site analysis (a total of 101 plots and plants) approximated the recommended minimum of five observations per estimated parameter (Shiple 2000) for our model. However, the sample of 12 observations for the site-year analysis fell well below this minimum, compromising our ability to test the model statistically. For this reason, we used the site-year analysis primarily to ask whether parameter estimates at this higher level fell within the 95% confidence interval of those derived from the within-site analysis.

TABLE 1. Total flower-hours of observation and rates of visitation, by study site and year; visitation is expressed as visits per open *Ipomopsis aggregata* flower per hour of observation.

Year	Site	No. focal units	Total flower-hours	Rate of visitation (no. visits·flower ⁻¹ ·h ⁻¹)			
				Pollinators†			
				Hummingbird	Bumble bee	Butterfly	Hawkmoth
1996	CC	6	8855	0.0378	0.0014	0.0002	0
	P	5	3781	0.0235	0	0	0
	T	6	2980	0.0289	0.0003	0	0
	V	6	5349	0.0222	0	0	0
1997	P	21	3094	0.0275	0	0.0003	0
	T	20	3226	0	0.0177	0	0
	V	20	1956	0	0.0020	0	0
	OH	30	11 411.5	0.0458	0.0002	0	0
	SN	31	17 779.6	0.0636	0.0026	0	0
1998	P	12	1694	0.0236	0	0	0
	T	12	1160	0	0	0	0
	V	12	1210	0.0256	0	0	0
1999	P	15	1579	0.0247	0.0013	0	0
	T	15	1278	0.0055	0.0078	0	0
	V	15	1076	0.0121	0	0	0
2000	P	9	468	0.0491	0	0	0
	T	12	1487	0.0578	0	0	0
	V	13	1572	0.0553	0	0	0
2001	P	29	2035	0.0143	0	0	0
	T	29	2321	0.0190	0.0043	0	0
	V	30	4313	0.0183	0	0	0.0114
2002	P	30	1505	0.0465	0	0.0020	0
	T	25	1863	0.0263	0	0.0011	0
	V	30	3211	0.1003	0	0.0009	0

Notes: The six study sites are meadows around the Rocky Mountain Biological Laboratory in western Colorado, USA. Ellipses (...) indicate missing data; observer scored all as "small insect."

† Hummingbird = Broad-tailed or Rufous Hummingbirds; Bumble bee = long-tongued *Bombus appositus* or *B. flavifrons* queens; Butterfly = swallowtail butterflies, *Papilio rutulus* or *P. gothica*; Hawkmoth = *Hyles lineata*.

‡ Nectar robber = short-tongued *Bombus occidentalis*.

§ Hoverfly = unidentified Syrphidae; Other fly = Bombyliidae, Muscidae, etc.; Solitary bee = unidentified Halictidae or Megachilidae; Wasp = unidentified Vespidae; Small insect = solitary bee, fly, or wasp.

Visitation rates to individual plants or plots were low enough for most visitor classes that added zeroes created positive kurtosis in the within-site analysis, which inflates the apparent significance of path coefficients (Shipley 2000). We minimized, but could not fully eliminate, kurtosis by $\ln(Y + 1)$ transformation of visitation rates, and by summing visitation rates by individual pollinator classes to obtain a less kurtotic measure of overall pollinator visitation.

RESULTS

The visitors of Ipomopsis aggregata

Over six sites and seven years we observed a total of 4174 visits to individual *I. aggregata* flowers during 555 h (85 204 flower-hours) of observation (Table 1). Hummingbirds were the most frequent visitors, contributing 78.7% of all visits observed, and 94.1% of 3492 visits by pollinators. Nectar robbers were the next most frequent visitors (8.2% of flower visits), followed by small insects (8.1%), long-tongued bumble bee queens (3.5%), hawkmoths (1.2%), and swallowtail butterflies (0.3%).

Average total visitation by pollinators was 0.041 visits per flower per hour of observation. Assuming a 12-h activity period for pollinators and that flowers spend on average 34 h in male and 37 h in female phase (M. V. Price, unpublished data), flowers received ~0.8 visits on average when stigmas were receptive and ~1.5 visits during their entire lifespan. This agrees with values from previous studies near the Rocky Mountain Biological Laboratory (RMBL; Colorado, USA) (Campbell et al. 1991, Mitchell 1993).

Variation in species composition of the visitor community

The spectrum of visitors varied considerably among years and sites (Table 1). For the three sites with seven years of data, factorial random-effects MANOVA (see Appendix A: Table A1) indicated significant variation among years in patterns of visitation by five visitor classes: hummingbirds, long-tongued bumble bees, Lepidoptera (butterflies + hawkmoths), nectar robbers, and small pollen-eating insects. Sites did not vary significantly overall in their visitor community, but did

TABLE 1. Extended.

Rate of visitation (no. visits-flower ⁻¹ ·h ⁻¹)					
Nectar robber‡	Pollen eaters§				
	Hoverfly	Other fly	Solitary bee	Wasp	Small insect
0.0146	0	0	0	0	0
0	0	0	0	0	0
0.0148	0	0	0	0	0
0	0	0	0	0	0
0	0.0100	0	0.0029	0	0.0129
0	0.0090	0	0.0015	0	0.0105
0	0.0061	0	0	0	0.0061
0	0	0	0	0	0
0	0	0	0	0	0
0	0.0077	0.0077	0.0012	0	0.0165
0	0	0.0017	0.0009	0	0.0026
0	0.0050	0	0	0	0.0050
0.0671	0.0044
0.0266	0.0031
0.0270	0
0	0.0363
0	0
0	0.0051
0	0.0221	0	0.0010	0	0.0231
0	0.0009	0.0004	0	0.0043	0.0064
0.0005	0.0218	0.0014	0.0021	0.0023	0.0513
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

differ in the magnitude of among-year variation (site \times year interaction).

Canonical discriminant analysis (CDA; see Appendix A) indicated that most of the variation among years was in the relative visitation rate by nectar robbers and long-tongued bumble bees vs. by hummingbirds, Lepidoptera, and pollen-eating insects. The primary axis of between-site variation separated sites on the basis of visitation by long-tongued bumble bees and, to a lesser extent, nectar robbers vs. other taxa. The two primary axes of site \times year variation reflected differences among sites in the extent of year-to-year variation in the visitor community.

The visitor community also varied significantly among Early, Middle, and Late periods of the season (see Appendix B). CDA indicated that periods were separated on the basis of nectar robber and hummingbird visitation contrasted with visitation by other taxa. Visitation by nectar robbers and hummingbirds was relatively high late in the season, whereas visitation by Lepidoptera, long-tongued bumble bees, and small insects was relatively high early in the season.

Variation in visitation rate by pollinators

Given the fluctuations in visitor fauna just described, it is not surprising that the total rate of visitation by pollinators also varied significantly. Year and site \times year interaction were significant, respectively explaining 7.7% and 8.5% of the variance, and site contributed

no significant variance beyond that from site \times year interaction. Patterns of variation were similar for nectar robbers and pollen eaters analyzed individually. Estimates of hummingbird visitation ranged from zero to 0.1003 visits-flower⁻¹·h⁻¹ (Table 1). Total rates of visitation by all pollinators varied somewhat less dramatically (eight-fold), from 0.0121 to 0.1012 visits-flower⁻¹·h⁻¹. These overall visitation rates translate into between 0.22 and 1.87 total pollinator visits to the average flower while stigmas are receptive, a range that could produce quite variable degrees of pollen limitation of seed production.

Relationship of visitation to stigma pollen loads: within-site path analysis

In general, the within-site path analysis supported hypothesized relationships among pollen delivery by different visitor classes, and between activity of these visitor classes and stigma pollen loads. The independence model provided a poor fit to the data ($\chi^2 = 31.69$, $df = 15$, $P < 0.01$), indicating that significant relationships exist among the measured variables. In contrast, the causal model of Fig. 1 fit the data reasonably well ($\chi^2 = 0.02$, $df = 1$, $P = 0.88$; Adjusted goodness-of-fit index = 0.999). Standardized regression coefficients indicated significant positive effects of visitation by hummingbirds and long-tongued bumble bees on stigma loads, and nonsignificant positive effects of visitation by Lepidoptera (Fig. 1). Based on untrans-

formed variates, the ratio of pollen delivery for bumble bees vs. hummingbirds was 2.23:1, in good agreement with the ratio of 2.96:1 obtained by directly measuring pollen deposition after single visits to virgin flowers (Mayfield et al. 2001). Robbers had negative (but non-significant) effects on rates of visitation by hummingbirds and Lepidoptera, and a smaller positive (also non-significant) effect on visitation by long-tongued bumble bees (Fig. 1). There were significant positive correlations between visitation rates by hummingbirds and Lepidoptera, Lepidoptera and pollen eaters, and hummingbirds and pollen eaters. LaGrange multipliers did not indicate that model fit would be improved by adding any effects left out of the model in Fig. 1, and Wald analysis did not suggest that fit would be improved by dropping any effects.

The full path model explained 9.6% of the within-site variance in stigma pollen loads. A simplified multiple-regression model that included only legitimate pollinators as the independent variables (hummingbirds, long-tongued bumble bees, and Lepidoptera) did nearly as well, explaining 8.2% of the variance and yielding path coefficients nearly identical to those in the full model. Excluding long-tongued bumble bees and Lepidoptera from the regression reduced the explained variance to 4.3%. Although this decline was not significant ($F_{2,97} = 2.05$, $P > 0.10$; Sokal and Rohlf 1995), the 50% reduction in R^2 suggests that long-tongued bumble bees and Lepidoptera play a tangible role in pollination of *I. aggregata* at the RMBL.

Regression analysis indicated that stigma pollen load (untransformed values) increased linearly with rate of visitation by pollinators summed. A quadratic model was significant overall ($F_{2,98} = 4.05$, $P = 0.02$), but whereas the linear term was significantly greater than zero ($b = 427$, $t = 1.83$, $P = 0.035$, one-tailed) the squared term was not different from zero ($b = 1329$, $t = 0.28$, $P = 0.78$, two-tailed), and the linear term by itself explained significant variation ($P = 0.006$).

*Relationship of visitation to stigma pollen loads:
site-year path analysis*

The small sample of site-year combinations ($n = 12$) compromised our ability to test the causal model at this higher spatiotemporal level. Indeed, although the hypothesized causal model fit the observed data at this level ($\chi^2 = 0.05$, $df = 1$, $P = 0.828$; Adjusted goodness-of-fit index = 0.970), so did the independence model ($\chi^2 = 10.49$, $df = 15$, $P = 0.766$), and none of the estimated regression coefficients or correlations was statistically significant. LaGrange multipliers did not indicate that model fit would be improved by estimating any fixed effects, nor Wald analysis by dropping any effects.

Whereas the site-year model fit is uninformative in itself, comparison with the within-site analysis reveals interesting differences (Fig. 1). Half the estimated parameters from the site-year analysis (7 of 14) lay out-

side the 95% confidence interval for coefficients from the within-site analysis (those in boldface), and some coefficients changed sign. For example, the site-year analysis suggests that hummingbird visitation has a negative effect, and pollen eaters a positive effect, on pollen loads; that robbers positively affect visitation by hummingbirds and Lepidoptera; and that hummingbirds are negatively, rather than positively, correlated with pollen eaters. These results suggest that factors not included in the causal model postulated in Fig. 1 are important in assessing relationships among floral visitors, and between visitation and pollen receipt, across years and sites.

Also in contrast to the within-site analysis, the simplified multiple-regression model that included only legitimate pollinators (hummingbirds, long-tongued bumble bees, and Lepidoptera) explained considerably less of the variance in stigma pollen loads among sites and years (4.0%) than did the full path model (12.7%), and resulted in path coefficients approximately half those of the full model. By these measures, leaving non-pollinators out of the model had a greater effect at the site-year than at the within-site level.

DISCUSSION

Our study confirms that pollination service varies considerably. Overall, the most common visitors to *Ipomopsis aggregata*, a classic "hummingbird syndrome" flower (e.g., Mayfield et al. 2001), indeed were hummingbirds. However, *I. aggregata* also received visitation from insects, including pollinators and larcenists (sensu Irwin et al. 2001). The relative abundances of the visitors to *I. aggregata*, including its "syndrome" pollinator, varied across years and within flowering seasons, and sites a few hundred meters apart differed in the magnitude of the temporal variation. This suggests that the relative contribution of any one visitor, including hummingbirds, to population dynamics or to selection on floral traits, is far from constant (compare Herrera 1988, Horvitz and Schemske 1990, Fenster and Dudash 2001).

Although visitation rates varied temporally and sites differed in this regard, our uniformly sunny open-meadow sites did not differ significantly, in contrast to patterns observed for relatively sedentary, microclimate-sensitive insects visiting flowers in more complex habitats (Herrera 1988, Horvitz and Schemske 1990). This relative lack of spatial variation does not appear, for hummingbirds at least, to be an artifact of the proximity of study meadows. In 1996 hummingbird visitation rates to *I. aggregata* were measured at a cluster of three meadows near Kebler Pass, at similar elevation but 16 km distant from the Rocky Mountain Biological Laboratory (RMBL; Colorado, USA) (A. K. Brody, unpublished data), using methods similar to those used in this study. Comparing those data to ours using a random-effects ANOVA indicated that neither cluster (Kebler Pass vs. RMBL), nor meadow nested within

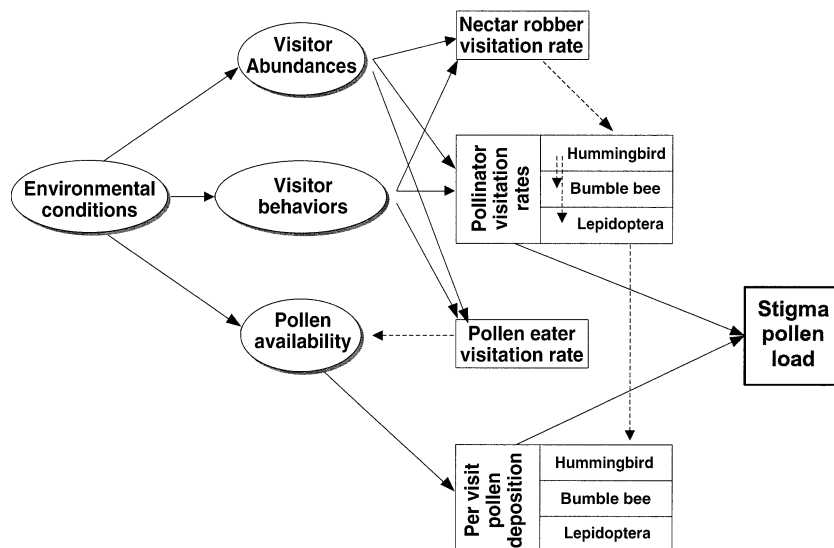


FIG. 2. Hypothesized causal model for variation in stigma pollen loads. Unmeasured latent factors are in oval frames; measured variables are in rectangular frames. Dashed lines indicate postulated negative effects. Environmental variables are hypothesized to affect pollen availability and component determinants of visitation by three functional visitor classes. Visitation rates and pollen availability determine visit rate and per-visit pollen deposition, which together determine stigma pollen loads.

cluster, contributed additional variance (REML [restricted maximum likelihood] variance component estimates = 0 in both cases) beyond residual plant-to-plant variation within meadows. It is not necessarily surprising that time seems to matter more than space on these scales, because mobile pollinators distribute themselves so as to approach ideal-free distributions in depletion of resources over short to moderate distances (e.g., Dreisig 1995). In contrast, temporal variation in environmental conditions is likely to affect component determinants of visitation rates on a regional scale. For example, variation in winter snowpack is likely to affect population densities of many flower-visiting insects, including bumble bees, whose queens overwinter in the soil and rely on insulating snow cover for survival. Variation in precipitation and temperature also might directly or indirectly influence nectar production (Kenoyer 1917, Huber 1956) and thereby floral preferences of pollinators and nectar robbers, as well as pollen production per flower (Lau and Stephenson 1993), and thereby per visit pollen transfer to stigmas (Wolfe and Barrett 1989). Annual climate fluctuation on a hemispheric scale is likely to influence overwinter survival of migratory hummingbirds and their success in southward (autumnal) and northward (vernal) migrations (e.g., Calder 1993). Furthermore, variable snowpack and summer precipitation translate into variable densities and phenologies of a succession of flowering plant species around the RMBL that compete with *I. aggregata* for pollination. These competitors not only affect visitor floral choices and deposition of foreign pollen on stigmas, but they also contribute resources in support of populations of the pollinators

of *Ipomopsis* (see Waser and Real 1979, Price and Waser 1998, Inouye et al. 2002).

Observed variation in the visitor community within single flowering seasons may be explained largely by seasonal patterns of visitor behavior and abundance at the RMBL (Waser 1976, Calder 1993, Irwin and Mallof 2002, Sharaf and Price 2004). Since the growing season is short at high elevation, most animals initiate reproductive activity soon after snowmelt. Hence, visitation by flies and solitary bees that collect pollen was concentrated at the beginning of flowering. Likewise, queen bumble bees forage early in the season to support nest establishment and production of workers, and cease foraging once workers emerge. Because workers are smaller than queens, with shorter proboscises, pollinating visits to *I. aggregata* by long-tongued *Bombus appositus* queens are concentrated early in the season. In contrast, the short-tongued workers of *B. occidentalis* cut holes in corollas to rob flowers, and their numbers peak late in the season. Broad-tailed hummingbirds also begin nesting early, but peak hummingbird activity occurs in midseason (late July to early August) when numbers are augmented by broad-tailed juveniles and the arrival of southward-migrating rufous and calliope hummingbirds.

To what extent does variation in visitation translate into variation in pollination service? Surprisingly, this question has been addressed in few studies of natural populations, even though it is widely recognized that visitation rate is at best a crude index of pollination service because pollinator species vary in per visit effectiveness (e.g., Beattie 1971, Primack and Silander 1975, Motten et al. 1981, Schemske and Horvitz 1984,

Wilson 1995). We were able to confirm that, when data were centered to remove site–year variation, stigma pollen loads of individual plants were positively related to observed rates of visitation by hummingbirds and pollinating insects. This result is consistent with a previous report (Engel and Irwin 2003) relating hummingbird visitation rate to pollen received by *I. aggregata* flowers at one site at the RMBL in 2001, and with an aviary study (Mitchell and Waser 1992) showing that pollen delivery to *I. aggregata* stigmas increases with the number of visits by hummingbirds. A positive link between visitation and pollen deposition, or an indicator of deposition, has been reported from a few other systems as well (Schemske and Horvitz 1984, Galen and Stanton 1989, Hodges 1995, Wilson 1995, Fishbein and Venable 1996, Jones and Reithel 2001).

A strong relationship between visitation and pollen receipt may not be universal, however. Pollen receipt may often be a decelerating function of the number of successive visits to a flower, and pollinators may dislodge pollen already deposited on stigmas (cf. Engel and Irwin 2003). The fact that the second-order term of quadratic regression was nonsignificant in our analysis suggests that visitation rates to *I. aggregata* are, on average, low enough that they fall within the linear portion of the dose–response function relating visitation to stigma pollen loads. Indeed, the maximum estimated visitation rates in our study were 1.87 visits per female-phase flower, whereas Mitchell and Waser (1992) found that pollen loads increased linearly up to five visits by hummingbirds.

Path analysis yielded quantitative estimates for the relative effects on pollen receipt of visitation by different pollinators and floral larcenists that agree with independent estimates derived by other means, including manipulative experiments (e.g., Irwin and Brody 1998, Mayfield et al. 2001, Engel and Irwin 2003). Hence an analysis of variation at the level of individual plots and plants confirms that our methods of observing visitors do in fact produce reasonable estimates of visitation rate and of the per visit effectiveness of different visitors in increasing (or decreasing) pollen delivery, either directly (pollinators, pollen eaters) or indirectly via effects on other visitors (nectar robbers).

One limitation of path analysis is that the magnitude of path coefficients and explained variance are dependent on which variables are included in the models. Causal factors not included contribute to unexplained variation in response variables, which typically is high in path models, such as ours, where dependent variables are affected by multiple-species interactions and multiple host traits (e.g., Schemske and Horvitz 1988, Conner 1996, Engel and Irwin 2003). In our case, a number of factors known to affect mechanics of pollen removal or deposition, such as variation among plants in flower morphology (e.g., Campbell et al. 1991, 1994), were

excluded from the model and likely contributed to unexplained variance in pollen receipt.

Whereas a causal model relating flower visitation to pollen delivery at the within-site level produced results that were immediately interpretable in light of past studies, the same model based on site–year means gave a different impression. For example, the latter model returned a negative effect of mean hummingbird visitation on mean pollen loads that was outside the 95% confidence interval of the within-site estimate. This result might be explained if hummingbird visitation were high, for whatever reason, in years and sites that were poor in pollen availability or in abundance of highly effective pollinators, such as long-tongued bumble bees. Indeed, across sites and years, visitation rates of hummingbirds were negatively correlated with long-tongued bumble bees (Fig. 1).

The discrepancy between within-site and site–year analyses suggests that the actual causal model that relates visitation rates to pollen receipt in *I. aggregata* is more complex than shown in Fig. 1, and should include environmental effects on visitation patterns and pollen availability. We venture such a model in Fig. 2. It postulates that environmental conditions exert direct effects on population-wide pollen availability by modulating flowering intensity and pollen production. Environmental conditions also directly affect abundances and foraging behaviors of individual species that determine per capita visitation rates. Together, these determine total visitation rates by functional visitor groups, such as pollinators, nectar robbers, and pollen eaters. Visitation patterns in turn affect either pollen availability (pollen eaters) or degree of stigma saturation (pollinators), both of which determine the average pollen deposition per pollinator visit. Effects of nectar depletion by hummingbirds and nectar robbers are suggested in negative direct effects on visitation rates by less efficient nectarivores. Even though Fig. 2 is simplified (for example, it subsumes diverse direct environmental effects into one latent variable), characterizing the paths in such a model, and testing overall model fit to observations, calls for a level of study beyond what has yet been attempted for any pollination system. We contend that it is useful to contemplate a model such as this precisely because it illustrates the factors that eventually must be explored to fully understand variation in pollination services to plants.

These results from *I. aggregata* should add to a growing appreciation that pollination services are variable, even on short to moderate temporal and spatial scales, and are sensitive to environmental conditions as well as presence of floral larcenists. This variation has implications for studies of pollinator-mediated selection on floral traits. Whereas studies of short duration at single sites may detect strong covariance of floral variation and pollination success, additional sources of variation across sites and years may weak-

en, strengthen, or even reverse this signal. Variation in pollination also has important potential implications for plant ecology, including conservation, insofar as pollination intensity influences seed production, seedling establishment, and plant population dynamics. In future papers we will evaluate these potential links between pollination and seed production, as well as between seed production and recruitment, survival, and reproduction of the subsequent plant generation.

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APPENDIX A

Three tables showing MANOVA results and canonical discriminant analysis of spatial and temporal variation in rates of visitation by five classes of visitor to *Ipomopsis aggregata* are available in ESA's Electronic Data Archive: *Ecological Archives* E086-112-A1.

APPENDIX B

Three tables showing MANOVA results and canonical discriminant analysis of seasonal variation in rates of visitation by five classes of visitor to *Ipomopsis aggregata* are available in ESA's Electronic Data Archive: *Ecological Archives* E086-112-A2.