

## EFFECTS OF POPULATION SIZE AND DENSITY ON POLLINATOR VISITATION, POLLINATOR BEHAVIOR, AND POLLEN TUBE ABUNDANCE IN *LUPINUS PERENNIS*

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Both the number and the density of flowering plants in a population can be important determinants of pollinator abundance and behavior. We report the joint effects of population size and density on pollinator visitation and pollination success for *Lupinus perennis* (Fabaceae). Focusing on five pairs of populations, we matched one small population (125–800 flowering plants) with one distinctly larger population (1000–3000 flowering plants). In these pairs, population size did not affect pollinator communities or pollination success. All measures of pollination success increased significantly with density. Only bee behavior (number of flowers probed per inflorescence) exhibited a significant interaction of size and density. Testing whether population sizes smaller than those in the paired populations might affect pollination, we gathered pollen tube samples from 14 unpaired populations (16–215 flowering plants). Combining these data with those from the paired populations revealed a significant decrease in pollination for smaller populations, indicating that effects of population size may be detectable only when populations smaller than a few hundred plants are sampled. We found that effects of density are consistent and much stronger than those of population size. Our results suggest that both size and density of natural populations should be considered in designing restoration and reintroduction programs for this threatened plant.

**Keywords:** *Bombus*, Fabaceae, conservation, plant-pollinator interaction, pollination, *Osmia*.

### Introduction

The number and density of flowering plants in a population can be important determinants of pollinator abundance and behavior (Sih and Baltus 1987; Kunin 1993, 1997; Rathcke and Jules 1993; Aizen and Feinsinger 1994a; Ågren 1996; Stout et al. 1998). From a pollinator's point of view, large and dense populations offer more resources (e.g., pollen or nectar) and are easier to detect from a distance. These factors often increase pollinator abundance and per-flower visitation rates in large populations (Jennersten 1988; Aizen and Feinsinger 1994b; Waites and Ågren 2004) and sometimes alter pollen quality (e.g., proportion of outcross pollen) because of their effect on pollinator movement patterns (Goulson 2000). Furthermore, the reduced interplant distances in dense populations should reduce travel costs, which may encourage visitors to stay longer in a patch or a population, therefore increasing visitation rate per flower in dense areas (Sih and Baltus 1987; Kunin 1993, 1997; Cartar and Real 1997; Bosch and Waser 2001). Density may also promote behaviors that increase self-pollination and otherwise alter pollen quality (Morris 1993; Karron et al. 1995). Because each pollinator taxon may re-

spond differently to these factors, the species composition of visitors may also vary with population size or density (Sowig 1989; Donaldson et al. 2002). Although the works cited above indicate that the size and density of plant populations may each affect pollination, few studies have looked at both in the same species (Kunin 1997; Molano-Flores and Hendrix 1999; Mustajärvi et al. 2001).

Changes in pollinator abundance, behavior, or species composition caused by plant population size and density may in turn affect plant reproductive success (Ågren 1996; Roll et al. 1997). Such responses have important conservation implications when they contribute to population declines (Rathcke and Jules 1993; Kearns et al. 1998; Spira 2001). However, this linkage is a poorly understood component of how anthropogenic changes in natural ecosystems lead to declining populations of many species.

*Lupinus perennis* (Fabaceae), a threatened, long-lived perennial herb, is currently listed as extirpated or vulnerable in parts of its range in the United States and Canada (NatureServe 2004) and is considered potentially threatened in Ohio (Ohio Division of Natural Areas and Preserves 1996). Habitat restoration efforts have targeted *L. perennis* because it is the only larval food source for the federally endangered Karner blue butterfly, *Lycaeides melissa samuelis* Nabokov (Haack 1993).

Cartwright (1997) found that reproductive success in *L. perennis* decreased as population size declined, even for populations that included many hundreds of individuals ( $150 \leq n \leq 800$ ). One potential explanation is that this decrease results from differences in pollination. In separate work (Shi 2004; X. J. Shi,

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H. J. Michaels, and R. J. Mitchell, unpublished data), we consider other potential causes of this pattern, such as reduced genetic variation and increased inbreeding. Here we address the effects of population size and density on pollinator behavior and pollinator abundance and the resulting abundance of pollen tubes in ovaries of *L. perennis*.

## Material and Methods

### Natural History

*Lupinus perennis* L. (Fabaceae) is a perennial legume that grows in sandy soils from Maine to Minnesota and south to Florida (Gleason and Cronquist 1991). This oak savannah species develops an extensive taproot from which short, vertical lateral shoots develop as the plant ages. The clumps that constitute individual plants can usually be distinguished by careful observation of leaf morphological details, phenology and flower color and (when necessary) by shallow excavation to determine stem origins and connections. In our study sites, the flowering season usually lasts ~4–6 wk, beginning in early May. Plants produce one to several dozen inflorescences, each bearing a total of ~35 flowers, of which ~20 may be open at any one time. The papilionaceous blue and white flowers are ~15 mm long and are produced in several whorls of five. Pollen is the only floral reward in *L. perennis*. Although *L. perennis* is partly self-compatible, full seed set requires pollinator visitation and receipt of outcross pollen (Shi 2004; Shi et al. 2005).

On the basis of our observations in the field, all of the common visitors to *L. perennis* contact reproductive parts and transfer pollen among flowers. These visitors primarily include bumblebees (*Bombus* spp.) and orchard bees (*Osmia* spp.). Ineffective visitors (including small bees, wasps, butterflies, and hummingbirds) were uncommon, and we do not consider them further. Pollination by effective visitors occurs when a bee braces her head against the banner petal and depresses the wing and keel petals with her metathoracic legs. This pumps pollen onto the ventral surface of the bee through a “syringe” mechanism and also extends the style out of the keel, allowing the mature stigma to receive pollen (Harder 1990).

### Populations

We studied *L. perennis* in five separate areas in northwestern Ohio and southeastern Michigan (Bernhardt 2000; Shi 2004). In each area, we located a pair of separate populations that were matched to be as similar as possible in physical features, proximity, and phenology (C. E. Bernhardt, R. J. Mitchell, and H. J. Michaels, unpublished data). We used a pair as a blocking variable to control for geographical variation in edaphic factors and pollinator availability. In each pair, prior surveys indicated that one population was distinctly smaller than the other (Bernhardt 2000; Shi 2004). On the basis of research by Cartwright (1997), our “small” populations typically had <800 reproductive plants and “large” populations had >1000; the density of flowering plants was similar for both members of each pair (Bernhardt 2000; Shi 2004). To avoid impacts on very small populations from our frequent visits, all of our paired study populations had at least 125 reproductive plants. We used the same pairings for both years of

this study. Populations within a pair were separated by 200–3000 m of lupine-free habitat, and pairs were in similar habitat (e.g., forest openings, disturbed areas). Locations included Kitty Todd Preserve (the Nature Conservancy), Oak Openings Metropark (Toledo Metroparks), Lou Campbell Nature Preserve (Ohio Department of Natural Resources), Petersburg Game Area (Michigan Department of Natural Resources), and private land.

### Pollinator Observations

In each population, we chose two pollinator observation plots (1 m × 2 m): one plot with few flowering lupines (sparse) and one with many (dense). Inflorescence density differed by more than threefold between these plots, from a high of  $21.4 \pm 1.0$  (mean inflorescence density/m<sup>2</sup> ± SE) for dense plots to  $6.0 \pm 0.4$  for sparse plots. In contrast, density did not differ between small and large populations ( $P > 0.9$ ). Within each pair of populations, we visually matched all four plots so that they had similar vegetative ground cover, light levels, canopy cover, and phenology. Furthermore, within a density level, floral density was similar for both plot types (i.e., floral abundance in all dense plots was similar to that in other dense plots). We used different 1 × 2-m plots in each year.

We recorded pollinator visitation to flowers in the observation plots once a week throughout the flowering season in both 1999 and 2000. We used two observers to simultaneously record visitation in both the large and small populations within a pair. Pairing observations in this way helps to control for effects of time of day, temperature, and other factors on pollinator visitation (Kevan and Baker 1983; Corbet 1990; Conner and Neumeier 1995; Waites and Ågren 2004). During each observation bout, we recorded all floral visitors in each of the two plots twice, during two separate 15-min periods, alternating between sparse and dense plots. For each visitor, we recorded the species and caste, the number of flowers visited on each inflorescence, and the total number of flowers visited in the plot. Before each set of observations, we recorded the number of open flowers in the plot. Before and after each observation, we recorded meteorological data such as light, temperature, and humidity, but analysis of those data did not clarify the patterns presented in this article (C. E. Bernhardt, R. J. Mitchell, and H. J. Michaels, unpublished data).

In 1999, we observed each population on four separate days during the flowering season, for a total of 16 15-min observations per population. In 2000, we observed each population on three separate days, for a total of 12 15-min observations per population (total  $n$  across years = 280 observation periods; because a cloudburst at the end of the day prevented completion of two observation periods, actual  $n = 278$ ). We used a stratified random order of sampling for each week, so that in each year, we observed each population at least once in the late morning, the early afternoon, and midafternoon.

### Indirect Measure of Pollinator Activity

The direct observations of pollinator behavior described above provide a detailed but time-restricted indication of overall pollinator activity. To better understand the pollination experience of flowers over their full lifetimes, in 2000, we also scored the number of pollen tubes in *L. perennis* ovaries. Because these

flowers accumulated pollinator visits and pollen tubes over their entire 5–8-d life span, they act as time-integrated indicators of pollination (Aizen and Feinsinger 1994a; Molano-Flores and Hendrix 1999). To assess pollination success, we scored the presence of pollen tubes in the ovaries of >20 randomly chosen focal plants in each of our 10 paired populations (because some plants were not flowering during collection periods, actual  $n = 18$ –27 plants per population). On two separate occasions (once in the first half of the flowering season and once in the second half), we collected up to three (usually two) late-stage flowers from each flowering focal plant in each population. We chose flowers that were old enough to senesce but had not yet formed fruits and were at a stage before flower abortion. If appropriate-aged flowers were not available, we did not sample the plant. We also recorded the number of reproductive stems of *L. perennis* within a 1-m radius of each focal plant (excluding the focal), as an index of local density. In total, we sampled 723 flowers on 228 plants. We refer to these as the “paired” samples. We fixed these flowers in FAA and later stained them for fluorescence microscopy, following Kearns and Inouye (1993; see Bernhardt 2000). To view the stained tubes, we used a 360-nm filter in an Olympus BX60 microscope at  $\times 100$ –200.

We analyzed two components of overall pollination success to serve as indices of pollinator activity in these samples: (1) proportion of flowers with at least one detectable pollen tube in the ovary and (2) mean number of pollen tubes in the ovary for flowers that had at least one pollen tube. These indices are multiplicative components of mean pollen tubes per flower (measure 1  $\times$  measure 2 = pollen tubes/flower) but allow separate consideration of what may be different biological causes of each (see Campbell 1989).

Development of this technique for *L. perennis* allowed us to study effects of population size in populations that were too small for use in our main study and were therefore not paired with populations of larger size. To do this, in 2000, we also sampled pollen tubes in flowers from a variety of these small populations in northwestern Ohio (Wood, Lucas, and Fulton counties). These 14 populations ranged from five to 210 reproductive plants in size and were in both disturbed and undisturbed habitats, including roadside, sparse forest, and forest openings (many were near the paired populations described earlier and under management by the same entities). In each population, we directly counted the number of plants flowering on that day. We refer to these as the “unpaired” samples.

From the unpaired populations, we collected flowers from each of up to 25 inflorescences along one to several 1-m-wide transects in each population. Whenever we encountered a flowering inflorescence on the transect that was >1 m from other sampled inflorescences (and therefore on another genetic individual), we collected up to two senescing flowers, as described earlier. Unlike for the paired populations, we sampled on only one day (in the middle of the flowering season) in each of the unpaired populations, and took only one or two flowers from each plant (=inflorescence). Total sample sizes were 350 flowers and 207 inflorescences.

### Analysis

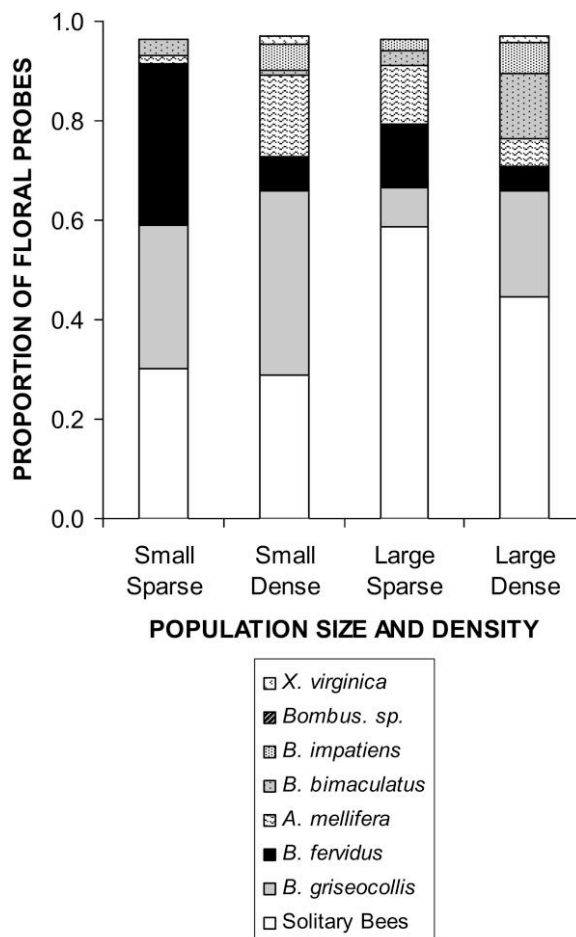
We analyzed these data using SAS 8.0 (SAS Institute 2000). In most analyses, we used fixed-effects ANOVA or ANCOVA,

under PROC GLM, with type III sums of squares. To normalize residuals, we natural-log-transformed numerical population sizes and square root-transformed the density of inflorescences within 1 m of focal plants for the pollen tube analyses.

## Results

### Pollinators

We recorded 271 pollinating insects in our observation plots during 278 15-min observations (69.5 h total across two years). The most common visitors, measured by probes per flower per 15 min (fig. 1), were bumblebees (six species of *Bombus* named in fig. 1, all native) and solitary bees (mostly *Osmia* species, some introduced and some native). Introduced honeybees (*Apis mellifera* L.) and native carpenter bees (*Xylocopa virginica* L.) were also present. Most bumblebees were workers (~67%),



**Fig. 1** Taxonomic composition of visitors to sparse and dense plots in large and small populations, based on proportion of the total visits per flower recorded during 15-min censuses. *Bombus* sp. includes *B. pennsylvanicus*, *B. affinis*, and individuals whose species identity could not be confirmed. On the basis of 100% of bees observed, percentage of pollinators is provided in each column (e.g., in small sparse plots, ~25% of the pollinators observed are solitary bees).

but this varied among species. For example, we saw almost exclusively workers of *Bombus griseocollis* (DeGeer) but only queens of *Bombus fervidus* (Fabricius). We could not distinguish most species of solitary bee on the wing. However, species-level identification of collected specimens indicate that the “solitary bee” category consists primarily of six species of *Osmia* (*Osmia atriveris* Cresson, *O. bucephala* Cresson, *O. distincta* Cresson, *O. lignaria* Say, *O. pumila* Cresson, *O. simillima* Smith), although it also includes several distinctive but less common species (e.g., *Andrena vieina* Smith and *Megachile melanophaea melanophaea* Smith).

We found no significant differences in the taxonomic diversity of bees (Shannon index based on visitation rate per flower for the groups listed in fig. 1) in our 10 paired populations as a function of population size ( $F_{1,37} = 0.07, P = 0.8$ ) or density ( $F_{1,37} = 3.46, P = 0.08$ ). Likewise, although there was a tendency for large populations to have more solitary bees (fig. 1), MANOVA shows no significant differences in the taxonomic composition of bees visiting lupine as a function of population size (Wilks’s  $\lambda = 0.78, P > 0.6$ ), density (Wilks’s  $\lambda = 0.79, P > 0.6$ ), or their interaction (Wilks’s  $\lambda = 0.75, P > 0.5$ ).

The visitation rate (probes per flower per 15 min) experienced by individual flowers varied significantly with plot density but not with population size class (table 1). Visitation rate per flower was on average 40% higher in dense plots (fig. 2; back-transformed mean = 0.097 probes/flower/h for dense plots, 0.048 for sparse plots). The significant “pair” effect re-

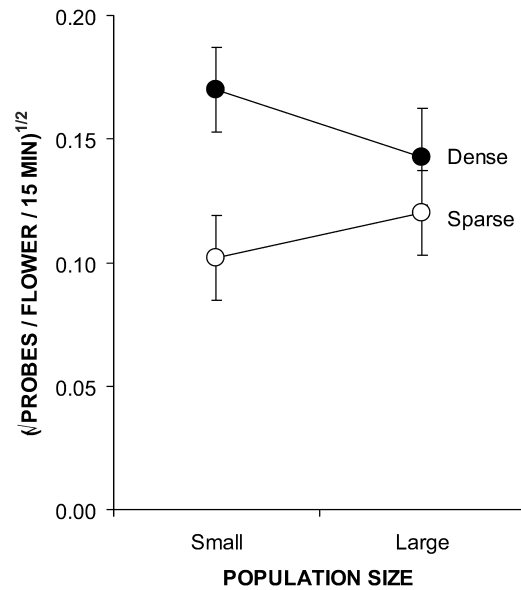


Fig. 2 Effects of population size and plot density on visitation rate experienced by flowers. Values shown are mean ± SE. Analysis is in table 1.

Table 1

ANOVA for Effects of Population Size, Inflorescence Density, Pairing, Year, and Week

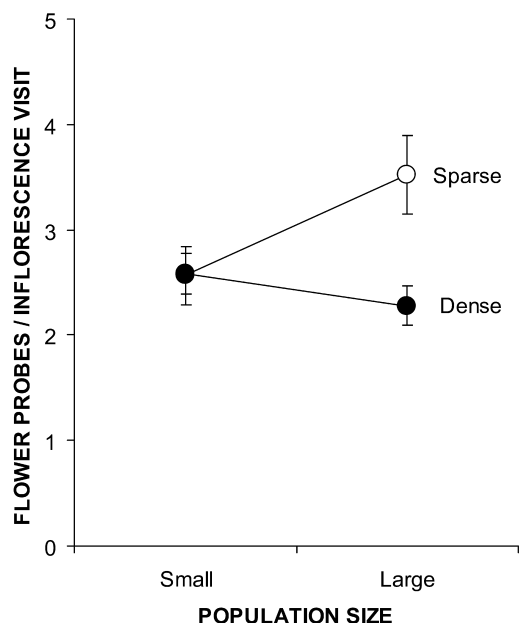
Source	df	MS	F	P
Size	1	.001	.07	.79
<b>Density</b>	1	<b>.139</b>	<b>6.88</b>	<b>.0096</b>
Size × density	1	.031	1.54	.22
<b>Pair</b>	4	<b>.083</b>	<b>4.10</b>	<b>.0035</b>
<b>Size × pair</b>	4	<b>.050</b>	<b>2.48</b>	<b>.0459</b>
Density × pair	4	.033	1.63	.17
Size × density × pair	4	.023	1.13	.34
Year	1	.001	.06	.81
Size × year	1	.010	.48	.49
Density × year	1	.054	2.68	.10
Size × density × year	1	.053	2.63	.11
<b>Pair × year</b>	4	<b>.202</b>	<b>10.01</b>	<b>&lt;.0001</b>
<b>Size × pair × year</b>	4	<b>.056</b>	<b>2.79</b>	<b>.0282</b>
Density × pair × year	4	.019	.93	.45
Size × density × pair × year	4	.043	2.14	.078
<b>Week (year)</b>	5	<b>.086</b>	<b>4.25</b>	<b>.0012</b>
Week (size × year)	5	.029	1.44	.21
Week (density × year)	5	.039	1.95	.09
<b>Week (pair × year)</b>	20	<b>.091</b>	<b>4.50</b>	<b>&lt;.0001</b>
Week (size × density × year)	5	.045	2.21	.056
Week (density × pair × year)	20	.019	.95	.52
<b>Week (size × pair × year)</b>	20	<b>.046</b>	<b>2.29</b>	<b>.0024</b>
Error	158	.020		

Note. Per flower visitation rates [(visits/flower/15 min)<sup>1/2</sup>] in 1 × 2-m plots. Model R<sup>2</sup> = 0.68, n = 278 15-min observation periods. Boldface indicates significance at P < 0.05.

ffects differences between pairs of as much as twofold. Although there was no significant effect of population size, the significant size × pair interaction reflects the fact that, in some pairs, large populations tended to have higher mean per-flower visitation rates than small populations, while in the others, the converse was true. Week-to-week variation in visitation rates was strong, mostly because of a peak in the middle of the flowering season in both years. Interactions of week with other factors were also significant, but there were no clear patterns in mean response. Although populations within a pair experienced similar visitation rates, this was not consistent across years; pairs with high visitation in one year often had low visitation in others, as indicated by the significant interaction of pair and year. Likewise, visitation rates for individual populations varied significantly among years (significant size × pair × year interaction). These significant interactions do not alter the general conclusions about the effects of size and density on visitation rate but serve to emphasize the large amount of variation intrinsic to plant-pollinator interactions.

We also analyzed our data with ANCOVA, using numerical population size estimates (based on 26–280 1-m<sup>2</sup> quadrat samples per population in 1999; Shi 2004), instead of the a priori size categories. This analysis again shows a significant increase in visitation with increased plot density (P = 0.048) but not with increased size (P = 0.25). No other factors or interactions were significant except for pair × year, reflecting up to threefold variation among years for some pairs.

Pollinators significantly altered their behavior in response to population size and the local density of flowering *Lupinus perennis* (fig. 3), in ways that might influence rates of geitonogamous self-pollination. Although there was a significant main effect of density (table 2), the most notable aspect of the response is the significant size × density interaction (table 2). Figure 3 shows that bees in sparse areas of large populations



**Fig. 3** Effects of population size and plot density on number of flowers probed per inflorescence visit by pollinators of *Lupinus perennis*. Values shown are mean  $\pm$  SE. Analysis is in table 2.

probed more flowers on an inflorescence before leaving than those in small populations, while the responses of bees in dense areas of large or small populations were nearly identical.

#### Indirect Measures of Pollinator Activity

As expected from direct observation of pollinator behavior, in our 10 paired study populations, we found that the proportion of flowers that had at least one detectable pollen tube increased significantly with local density (within 1 m) of conspecific flowering stems (fig. 4; table 3), approximately doubling as the number of stems within 1 m increased from none to more than 20. In contrast, there was no significant relationship between local density and the abundance of pollen tubes in flowers that had at least one tube over the same range, although this response variable varied slightly but significantly among pairings. The other main effects and interactions in both analyses were not significant. Analysis of pollen tubes per flower (=proportion of flowers with tubes  $\times$  tubes/pollinated flower) also shows a significant effect of density but not of size (results not shown).

We then used population means to analyze the pooled pollen tube data, combining the 10 populations of five pairs and the 14 unpaired populations (we could not assess effects of local density for this analysis because those data were not available for the unpaired populations). Proportion of flowers with tubes increased significantly with population size (table 4) in each group. For both groups, the proportion increased at about the same rate (interaction of group and size was not significant), but the unpaired populations had greater pollination success for a given size. The two groups in figure 5 were sampled differently and cannot be directly compared with each other. The paired populations were sampled throughout the flowering season, while the unpaired populations were sampled

only once, at peak flowering and pollination. Thus, the difference in means for these groups is not meaningful. In contrast to the results for proportion of flowers with tubes, pollen tubes per pollinated flower did not change significantly with size or any factor in either group (table 4; fig. 5).

## Discussion

We found that pollination of *Lupinus perennis* increased with both population size and population density. However, the effects of density were stronger and more consistent than those of population size. Density significantly affected not only pollinator visitation rates but also pollinator behavior and the proportion of ovaries with pollen tubes. In contrast, population size had detectable effects only on the proportion of ovaries with pollen tubes and then only when populations smaller than 215 flowering plants were included in the analysis.

#### Joint Effects of Population Size and Density

Few other studies have looked at the joint effects of population size and density on pollination, and none of those tested statistically for interactions between these main effects. Kunin (1997) found strong effects of density and little or no effect of population size on several measures of pollinator service for experimental populations of *Brassica kaber*. He also found hints of a synergistic effect for visitation (higher visitation in denser areas of large populations). Molano-Flores and Hendrix (1999) studied natural populations of *Anemone canadensis* and found that effects of population size and density on stigmatic pollen loads and reproduction were erratic across years. Experimental manipulations of *Lychnis viscaria* by Mustajärvi et al. (2001) revealed effects of both size and density but no hint of an interaction. Our work agrees with these and other studies, indicating that pollination is facilitated by density of conspecifics (Kunin 1997) and provides some indication that the effects of density and of size may be largely independent of one another.

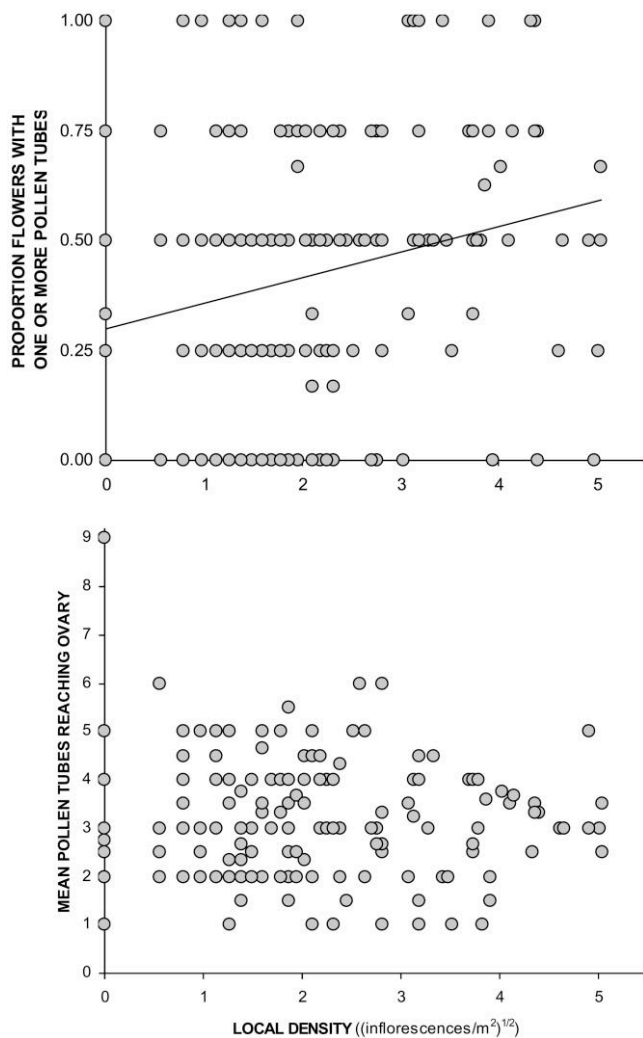
A striking result of our work in paired populations is that both pollinator visitation rate and proportion of flowers with pollen tubes increased with local density but not with population size. One possible explanation for this pattern is that it is an

**Table 2**

**ANOVA for Effects of Population Size, Local Density, and Population Pair on Number of Flowers Probed per Inflorescence by Insect Visitors to *Lupinus perennis***

Source	df	MS	F	P
Size	1	2.534	1.65	.20
<b>Density</b>	1	<b>8.519</b>	<b>5.56</b>	<b>.020</b>
<b>Size <math>\times</math> density</b>	1	<b>8.869</b>	<b>5.79</b>	<b>.018</b>
Pair	4	2.400	1.57	.19
Size $\times$ pair	4	1.211	.79	.5
Density $\times$ pair	4	2.246	1.47	.22
Size $\times$ pair $\times$ density	4	.739	.48	.7
Error	115	1.532		

Note. Model  $R^2 = 0.193$ ,  $n = 135$  observation periods (those in which floral visitors were present). Boldface indicates significance at  $P < 0.05$ .



**Fig. 4** Effects of local density on indirect measures of pollination success.  $n = 228$  focal plant means. Analysis is in table 3. Regression line is shown when significant.

artifact resulting from the fact that there is a larger difference between density categories than between size categories. This is unlikely because inflorescence density differed by  $\sim 3.5$ -fold, while population size differed by nearly the same multiple (2.7), so other factors probably contribute. Another possible explanation for why density effects were more pronounced than size effects is that pollinators may base most foraging decisions on the 2–3- $m^2$  scales over which we assessed density rather than the several-hundred-meters-square scales over which we scored population size (see Klinkhamer et al. 2001; Lennartsson 2002). We have no way of assessing this scenario with existing data.

A third possible explanation is that we studied populations larger than the threshold for effects to be detectable, while the range of densities encompassed the range of maximal effect. This seems feasible; constraints on our design prevented us from studying paired populations smaller than 125 flowering plants, and the pollen tube samples from the unpaired populations suggest that examination of smaller populations is necessary to de-

tect a significant effect of population size on pollination. Further studies involving direct comparisons of pollinator visitation as well as pollen tubes in both very small and larger populations would be valuable. In particular, they would help to determine whether this response is the result of changes in visitation rate, pollen transfer efficiency, pollinator species composition, or other factors. However, assessment of pollen tubes in ovaries is logistically much quicker and easier than direct observation of pollinators, and for many conservation purposes, it may not be necessary to determine the mechanisms responsible for changes in pollination service.

Dense patches within populations experienced increases in pollinator visitation rates (probes per flower per hour) and changes in patterns of movement (flowers probed per inflorescence). The former response may indicate intraspecific facilitation for pollinator visitation (Thomson 1982; Rathcke 1983; Sih and Baltus 1987; Feinsinger et al. 1991; Laverly 1992; Moeller 2004). Facilitation at high density can result if bees prefer dense areas and make foraging decisions based on local density (on a scale of a few square meters). This is consistent with foraging theory (Pyke et al. 1977), which predicts that bees will choose to forage in dense patches because of an expectation of reduced interinflorescence travel costs, and with studies of bee perceptual abilities showing that targets must cover  $5^\circ$  or more of visual angle for bees to detect and respond to them (Giurfa and Lehrer 2001). Regardless of their cause, such changes in visitation rate with local density may affect plant reproductive success by reducing the amount of outcross pollen received by flowers (Jennersten and Nilsson 1993; Petanidou et al. 1995; Ågren 1996; Kunin 1997; Bosch and Waser 1999).

In our study, bees probed more flowers on inflorescences in sparse areas of large populations but not in those of small populations (fig. 3). Although foraging theory (Pyke et al. 1977) predicts that visitors should probe more flowers per inflorescence in sparse patches (because of greater flight costs; see also Heinrich 1979), this did not occur in our small populations. The reasons for this strongly significant but unexpected behavioral change are unclear, but they may again involve pollinators making decisions on small spatial scales (see also Klinkhamer and van der Lugt 2004). Regardless of their cause, such changes in number of flowers probed on an inflorescence may affect reproductive success by increasing geitonogamous self-pollination. The consequences of geitonogamy for *L. perennis* are not known, although there is substantial inbreeding depression in this species (Shi et al. 2005; Michaels et al. 2008), and work with other species indicates that an increase in geitonogamous moves can increase the selfing rate (Karron et al. 2004). Although outcrossing rates are often positively correlated with both densities and population size (Barrett and Husband 1990; Van Treuren et al. 1993; Karron et al. 1995), few studies have tested for their joint effects on mating patterns (see Herlihy and Eckert 2004).

#### *Indirect Measures of Pollinator Activity*

Our pollen tube samples indicated that pollination service increased with population size and density, with the density response being much stronger and easier to detect. This response is consistent with our direct observations of pollinators in the paired populations. Several other studies have found the

Table 3

ANOVA of Effects of Population Size (Large vs. Small), Population Pairing, and Local Density on Proportion of Flowers with Pollen Tubes and Mean Pollen Tubes Reaching the Ovary in Flowers That Had Detectable Pollen Tubes

Source	Proportion of flowers with pollen tubes <sup>a</sup>				Mean pollen tubes/pollinated flower <sup>b</sup>			
	df	MS	F	P	df	MS	F	P
Size	1	.031	.33	.6	1	1.174	.85	.4
Pair	4	.064	.69	.6	4	4.877	3.51	.009
Density	1	<b>1.066</b>	<b>11.6</b>	<b>.0008</b>	1	2.045	1.47	.2
Size × pair	4	.0445	.49	.7	4	2.142	1.54	.2
Density × size	1	.014	.15	.7	1	2.407	1.73	.2
Density × pair	4	.054	.58	.7	4	2.989	2.15	.08
Density × size × pair	4	.021	.23	.9	4	2.870	2.07	.09
Error	205	.092			149	1.388		

Note. Local density = (density of inflorescences within 1 m)<sup>1/2</sup>. Boldface indicates significance at  $P < 0.05$ .

<sup>a</sup>  $R^2 = 0.101$ ,  $n = 224$  focal plant means.

<sup>b</sup>  $R^2 = 0.154$ ,  $n = 169$  focal plant means.

same patterns for one or both of these factors (Feinsinger et al. 1991; House 1993; Molano-Flores and Hendrix 1999).

Although the proportion of flowers receiving any pollen tubes increased with local density and population size, the number of pollen tubes reaching the ovary did not. This pattern is consistent with our understanding of pollinator behavior and pollen transfer abilities. First, there is likely to be high variance in the number of probes received by lupine flowers because of the nature of bee behavior. Given ~12 h of bee activity per day and a flower life span of ~5 d, our observed visitation rates translate to a mean total of six probes per flower over a flower's lifetime in the dense plots versus three in the sparse plots. Pollinator arrivals at individual flowers frequently approximate a Poisson distribution (Burd 1994; Dreisig 1995), so there should be substantial variation among flowers in the total number of flowers receiving two or more visits, with some receiving no visits and others receiving many (see Sih and Baltus 1987). Second, other work in this system (Hevner 2001) indicates that two or three floral probes can fully pollinate flowers. When the mean visit rate increases (approximately doubling for high-density plots in our study), the major effect would therefore be to provide a first or second visit to some flowers (increasing the proportion of flowers with pollen tubes) but not necessarily to increase the mean number of pollen tubes. One possible interpretation of there being a response in proportion but not mean pollen tubes is that as a lupine population declines in size, it is less likely to be discovered by bees, causing a larger fraction of flowers to never be visited during their lifetime. Declines in population size may also be accompanied by fragmentation, which has been shown to decrease visitation frequency of *Bombus verteranus* (Goverde et al. 2002). A meta-analysis of habitat fragmentation studies also demonstrates that fragmentation has a large negative effect on plant reproduction (Aguilar et al. 2006).

For both pollen tube variables, there was distinctly more variation for the unpaired samples than for the paired samples. This is probably a result of smaller sample size (number of ovaries sampled per plant; one sample of two ovaries for unpaired samples, and two samples for paired samples). However, this might also have a biological basis, perhaps indicating that the larger (paired) populations might be more reliably found and

returned to by bees or that smaller populations are in habitats that are somehow more variable in their suitability to bees.

#### Pollinator Visitation

In general, bumblebees were the most common visitors to *L. perennis* (fig. 1). However, because of substantial variation among populations and patches in abundances of different floral visitors (C. E. Bernhardt, R. J. Mitchell, and H. J. Michaels, unpublished data), in some sites *Bombus* was not the primary visitor of lupine. This variation may result from the restricted foraging ranges of smaller pollinators (*Osmia* spp.), individuals of which appeared to nest adjacent to some plots (C. E. Bernhardt, R. J. Mitchell, and H. J. Michaels, personal observation) and repeatedly returned to visit them. In contrast, *Bombus* forage more widely (L. W. Macior, personal communication) and probably did not return to individual plots during the period of our observations. Differences in the abundance of *Apis mellifera* may reflect abundance of hives in the surrounding agricultural landscape. We consider this unlikely to be important in our results because the highest abundance of *Apis* (fig. 1) occurred in plots separated by only 5 m from those with the lowest, a negligible distance for foraging bees. Differences in behavior among pollinator species such as those we

Table 4

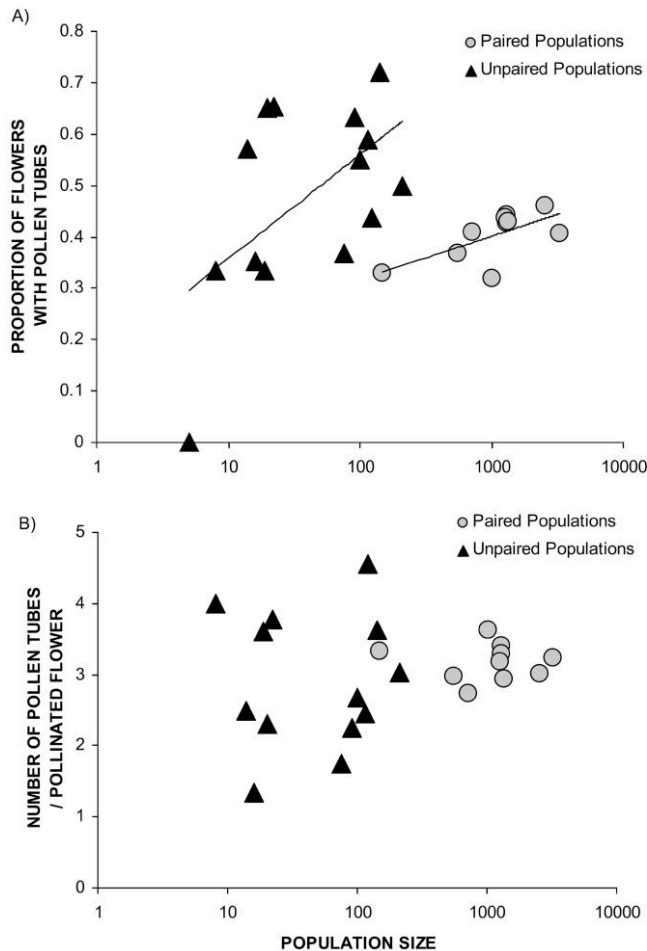
ANOVA of Effect of Numerical Population Size and Sampling Group (Paired Populations vs. Unpaired Populations) on Two Indirect Measures of Pollination Success

Source	Proportion of flowers with pollen tubes <sup>a</sup>				Mean pollen tubes/pollinated flower <sup>b</sup>			
	df	MS	F	P	df	MS	F	P
ln(size)	1	.07728	<b>4.61</b>	<b>.04</b>	1	.0021	.00	.9
Group	1	.00001	.00	.9	1	.0260	.04	.8
Interaction	1	.01268	.76	.3	1	.0093	.02	.9
Error	20	.33457			19	.5960		

Note. Boldface indicates significance at  $P < 0.05$ .

<sup>a</sup>  $R^2 = 0.359$ .

<sup>b</sup>  $R^2 = 0.034$ .



**Fig. 5** A, Proportion of flowers with pollen tubes. The separate regressions for each group are indicated by lines. Regression equation for paired populations is population mean proportion of flowers with pollen tubes =  $0.477 + 0.037 \times \ln(\text{size})$ ; intercept not significant (NS), size  $P = 0.04$ ,  $\beta = 0.659$ . For unpaired populations, population mean proportion of flowers with pollen tubes =  $0.154 + 0.0882 \times \ln(\text{size})$ ; intercept NS, size  $P = 0.04$ ,  $\beta = 0.559$ ,  $n = 10$  population means for paired populations, 14 population means for unpaired populations. On average, 18 plants contributed to each population mean. B, Mean pollen tubes per pollinated flower. Regression analyses of these data separately and pooled were NS ( $P > 0.4$ ), so regression lines are not shown.  $n = 10$  population means for paired populations, 13 population means for unpaired populations (one unpaired population had no flowers with pollen tubes). On average, 18 plants contributed to each population mean.

observed may have important consequences for plant reproductive success and mating patterns, but at this point, we do not have enough data to address those possibilities.

We found no significant effect of population size or density on the taxonomic diversity of pollinators, although this varied substantially among populations (C. E. Bernhardt, R. J. Mitchell, and H. J. Michaels, unpublished data). This lack of a consistent effect of size or density is perhaps not surprising because generalist bees are the primary visitors of *L. perennis*. Only a few studies have tested for effects of population size or density on species composition, and although Sowig (1989) found sig-

nificant effects of population size, most other studies were like ours in finding either weak (Donaldson et al. 2002) or no effects (Cane 2001; Mustajärvi et al. 2001; Donaldson et al. 2002; Yates et al. 2007).

### Conservation Implications

Because of its role as the sole host plant for a federally endangered butterfly, populations of *L. perennis* have benefited from ecological studies seeking to improve its growth and reproductive performance through habitat restoration (Smallidge et al. 1996; Forrester et al. 2005; Chan and Packer 2006). However, if butterfly reintroduction and restoration are to have long-term success, it is essential to understand the processes that lead to decline of the host species. Our studies show that pollinator visitation and pollination in this system were sensitive to within-population variation in density and reduction in population size to below a few hundred. These data from a not-yet-endangered plant serviced by relatively unspecialized pollinators suggest that, during species declines, density effects may compromise pollinator service before population size effects arise. Human impacts on natural populations may change the distribution of resources for pollinators long before plant populations show declines in demographic rates. Perhaps reductions in habitat quality that reduce plant growth and population density lead to changes in pollinator service that become the catalyst for increased geitonogamy, consistent with the increase in flowers visited per inflorescence in sparse areas of large populations seen in our study. Closer examination of how pollinator visitation and foraging patterns change with density is needed to further understand the contribution of initial changes in pollinator service to the dynamics of reproductive declines. Pollen tube samples appear to be a logistically more tractable way to assess pollination success than direct observations. Thus, they may have potential as an “early warning system” (Aizen and Feinsinger 1994a). All may appear to be well from the point of view of the bee populations and apparent plant population stability, but the plants may still be affected by changes in pollinator services. Finally, our analyses also found that population size influenced the proportion of ovaries with tubes for populations of  $<200$ . Thus, once populations become relatively small, declines in the numbers of flowers setting fruit may further limit population growth. Furthermore, because rare species often occur in populations of 200 or less, our data suggest that both size and density of new or augmented natural populations should be considered in the design of restoration and reintroduction programs.

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