

PLANT GENERALIZATION ON POLLINATORS: SPECIES PROPERTY OR LOCAL PHENOMENON?¹

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Despite recent increased interest in the frequency and evolutionary consequences of generalization in plant–pollinator systems, little is known on whether plant generalization on pollinators actually is a species-level trait. This paper addresses the following questions for the insect-pollinated shrub *Lavandula latifolia*: (1) Are different populations of this pollinator-generalist plant similarly generalized? (2) Within a highly generalized population, are all plants similarly pollinator-generalists? Comparable values for richness in pollinator species were obtained from individual- or population-specific rarefaction curves as the projected number of distinct pollinator species implicated in 100 flower visits (S_{RAR100}). Simple counts of pollinator species recorded per individual or population (S_{OBS}) were weakly or nonsignificantly correlated with corresponding S_{RAR100} figures and closely correlated with flower visitation frequency. The pollination system of *L. latifolia* was highly generalized at the regional level, but populations differed greatly in pollinator species richness (S_{RAR100}). Within the population intensively studied, individual plants had quite variable degrees of generalization, comparable in magnitude to variation among populations. It is concluded that generalization was not an invariant, species-level property in *L. latifolia*. Furthermore, pollinator diversity estimates based on S_{OBS} data may be heavily contingent on aspects related to both research design (sampling effort) and biological phenomena (variation in pollinator abundance or visitation rates), which may either mask or distort underlying ecological patterns of interest.

Key words: annual variation; geographical variation; individual variation; *Lavandula latifolia*; pollinator diversity; pollinator generalization; rarefaction curves; species richness.

There has been a recent upsurge of interest in the generalization-specialization gradient exhibited by animal-pollinated plants (Johnson and Steiner, 2000). This upsurge has been partly due to increasing recognition that, although specialized pollination systems undoubtedly exist (Pellmyr, 2002), generalist pollination systems seem to predominate in nature, with most species being pollinated by taxonomically diverse arrays of pollinators (Herrera, 1996; Waser et al., 1996; Olesen, 2000). Investigations on the origin, persistence, frequency of occurrence, and evolutionary consequences of generalist pollination systems are of interest from both ecological (e.g., in relation to the topology and structure of community-wide plant–pollinator interaction networks; Memmot, 1999; Dicks et al., 2002; Olesen and Jordano, 2002; Bascompte et al., 2003) and evolutionary viewpoints (e.g., in relation to the role played by pollinators in the evolution of floral traits and diversification of flowering plants; Herrera, 1996; Waser et al., 1996; Armbruster et al., 2000; Waser, 2001; Gómez, 2002).

Despite increased interest in generalist pollination systems, two critical aspects have never been concurrently investigated for any plant species. (1) Is pollinator generalization a species-level trait or, in other words, do all populations of a generalist species have roughly similar levels of generalization on pol-

linators? (2) Within a population of a generalist plant, are all individuals similarly pollinator-generalized? These questions are related to the broader issue of whether ecological specialization is an inherent property of a species, an attribute of local populations, or a trait of individual organisms (Fox and Morrow, 1981). In the case of herbivorous insects, for example, polyphagous species may be made of populations having relatively narrow host ranges (Fox and Morrow, 1981; Thompson, 1994), and a relatively polyphagous population may be made up of either generalist or specialist individuals (Bernays and Minkenberg, 1997; Bernays and Singer, 2002). Likewise, plant species with generalist pollination systems may be made of populations having relatively specialized pollinator assemblages, and generalist populations may be composed of variable mixtures of generalist and specialist individuals. These different scenarios entail contrasting evolutionary consequences (e.g., Thompson, 1994, 1999; Gomulkiewicz et al., 2000), yet empirical information on these aspects of the ecology of generalist insect-pollinated plants is virtually nonexistent.

In this paper, I will address these questions for *Lavandula latifolia* Medicus (Lamiaceae), a shrub pollinated by a very diverse insect assemblage (Herrera, 1988). Addressing these questions raises the critical issue of how to properly compare pollinator diversity among populations and among individuals within populations, an aspect that has received surprisingly little attention to date. A secondary objective of this paper is thus to propose a method based on rarefaction procedures (Gotelli and Colwell, 2001) to obtain truly comparable, standardized measurements of pollinator diversity. Recent analyses of the degree of specialization of plant–pollinator interactions have frequently used the number of visitor taxa as a measure of generalization (e.g., Waser et al., 1996; Olesen and Jordano, 2002; Kay and Schemske, 2004), yet these studies have generally proceeded without considering the possible pitfalls involved in comparing pollinator diversity estimates based on

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raw species counts (but see Ollerton and Cranmer, 2002). In the next section, I briefly introduce some of these pitfalls and justify the approach adopted in this paper.

How to measure plant pollinator generalization?—Plant generalization on pollinators may be variously defined (Herrera, 1996; Waser et al., 1996; Armbruster et al., 2000; Johnson and Steiner, 2000; Olesen, 2000; Gómez, 2002; Olesen and Jordano, 2002; Nakano and Washitani, 2003; Kay and Schemske, 2004). Here it is defined in relation to the number of pollinator taxa involved in the interaction, as distinct from morphological or evolutionary generalization. Thus defined, quantifying the degree of plant generalization on pollinators boils down to evaluating pollinator diversity (Ollerton and Cranmer, 2002). This means, on one side, that the task is subject to the same deceptive simplicity, and prone to the same sampling biases and pitfalls, long known to complicate estimates of species diversity of plant and animal communities (e.g., Magurran, 1988; Gotelli and Colwell, 2001). Comparisons based on crude lists of pollinator taxa that do not correct for the influence of differential sampling effort or pollinator abundance may be fatally flawed (Ollerton and Cranmer, 2002), in the same way and for the same reasons as are comparisons of plant community species richness that neglect the influence of variable number of quadrats or differential abundance of individuals (Magurran, 1988; Gotelli and Colwell, 2001). On the positive side, however, approaching the study of pollinator generalization as a particular case of species diversity assessment has some advantages. Once the set of entities on which diversity measurements are to be taken is explicitly defined, then methods devised for measuring the diversity of ecological communities may be readily imported for use with pollination data.

Generalization being a concept pertaining to the plants, it is the plant's "perception" of pollinator diversity, not the ecologist's, that should prevail when measuring pollinator diversity. I suggest that a plant's perception of pollinator diversity should be assessed on the "population" of entities made up of flower visitation events, rather than on the set of biological individuals (pollinators) interacting with the plant. In other words, I propose that the relevant magnitude to quantify pollinator generalization is the diversity of *flower visits*, rather than that of *floral visitors*. The visitor-centered approach, which is closer to the ecologist's perception of pollinator diversity, has been adopted frequently (e.g., Herrera, 1988; Mahy et al., 1998; Memmott, 1999; Dicks et al., 2002; Meléndez-Ramírez et al., 2002). Its results, however, will generally provide a distorted picture of a plant's perception of pollinator diversity because pollinator taxa differ extraordinarily in one or more of the following parameters: (1) number of flowers visited per time unit, (2) per-visit probability of effecting a pollen transfer event, and (3) average quality of pollen transfers effected (e.g., Schemske and Horvitz, 1984; Herrera, 1987, 1989; Thompson and Pellmyr, 1992; Gómez and Zamora, 1999; Thompson, 2001). Although it admittedly neglects aspects (2) and (3), a visit-centered approach to measuring pollinator diversity represents a significant improvement over the visitor-centered one in that it at least incorporates information on (1), the number of flowers visited per unit time. Reasons for adopting a visit-centered approach to measure pollinator diversity are essentially the same as those underlying the customary use of the proportion of flowers visited by different taxa to quantify the differential strength of inter-

action of one plant with its different pollinators (e.g., Gómez and Zamora, 1999; Potts et al., 2001; Kay and Schemske, 2003). Furthermore, the visit-centered approach is conceptually linked to the visitation-rate component of pollinator importance, as implied in Stebbins' (1970, 1974) "most-effective-pollinator principle" and made explicit, among others, by Armbruster (1988), Herrera (1989), and Armbruster et al. (2000).

Provided that (1) information on flower visitation events by pollinators is collected using an adequate sampling protocol based on random independent samples and (2) flower visitation events are categorized as to the taxonomic identity of the pollinator involved, then quantitative measurements of pollinator diversity useful for comparative purposes may be obtained by the same procedures used in the study of community diversity. Adoption of this approach will make clear that pollinator diversity measurements are prone to suffer from the same pitfalls and sampling biases long known to affect community diversity studies (Magurran, 1988; Gotelli and Colwell, 2001). Of all these, the potentially misleading influence of pollinator abundance (from the plant's perspective, i.e., as reflected in flower visitation probabilities) on estimates of pollinator diversity must be singled out, because its influence is both more elusive and more difficult to counteract than that of differential sampling effort. Without adequately accounting for differential pollinator activity or abundance, even comparisons of pollinator species richnesses based on similar sampling efforts (e.g., similar duration of watching intervals, observation days, or number of pollinator censuses) may still largely reflect differences in pollinator abundance rather than pollinator diversity itself. For this reason, in this study I will use sample-based rarefaction curves scaled to number of "individuals" (sensu Gotelli and Colwell, 2001) to estimate pollinator species richness of *Lavandula latifolia* populations and individual shrubs. Rarefaction is a statistical method first proposed by Sanders (1968) to overcome the problems involved in comparisons of community samples based on different sample sizes. It allows for estimation of the number of species (s) expected in a random sample of n individuals taken from a larger collection made up of N individuals and S species (Krebs, 1989). Species richness was preferred as a measure of pollinator diversity over, e.g., diversity indices, because it is the simplest way to describe diversity, has good discriminant ability (Magurran, 1988; Gotelli and Colwell, 2001), and has been used previously to measure pollinator diversity (e.g., Waser et al., 1996; Ollerton and Cranmer, 2002). It must be noted, however, that focusing on pollinator species richness alone neglects the possible significance of the evenness (or equitability) component of pollinator diversity, an aspect that would deserve consideration in future studies.

MATERIAL AND METHODS

Study plant—*Lavandula latifolia* is a low evergreen shrub common in the understory of open mixed woodlands at middle elevations in the eastern and southeastern Iberian Peninsula. The composition of the pollinator assemblage, the relation of the plant with pollinators, and other aspects of its reproductive biology have been described in detail elsewhere (Herrera, 1987, 1988, 1995, 2000). Flowering takes place in summer (July–September). Each shrub may produce up to a few thousand flowers per flowering season, with dozens to a few hundreds of flowers simultaneously open on the same plant. Flowers are hermaphroditic, have pale-blue tubular corollas and are self-compatible, but <4% of flowers set fruit in the absence of pollinators. In the Sierras de Ca-zorla and Segura study region (see next), >100 bee, fly, and butterfly species

have been recorded as pollinators of *L. latifolia* flowers, which clearly makes this species an outstanding example of generalist pollination.

Study sites and methods—Data used in this paper were collected during 1991–1997 at 15 *L. latifolia* populations located in the Parque Natural de Cazorla-Segura-Las Villas, Jaén Province, southeastern Spain. Locality names, geographical coordinates, and elevation of study sites are given in Table 2.

Differences among individual shrubs in pollinator diversity were studied in 1991 at a single *L. latifolia* population growing around Arroyo Aguaderillos, at 1160 m elevation. Pollinator flower-visitation data were collected from 15 flowering shrubs between 20 July and 10 August. The two most distant plants were 30 m apart. Pollinators were censused on these plants from dawn to dusk throughout the study period according to a randomized sampling design. Each census lasted for 5 min, when I closely watched the activity of pollinators at one of the marked shrubs. All flower visitors were identified to species, and information from previous studies (Herrera, 1987; C. M. Herrera, unpublished observations) was used to ascertain their status as true pollinators. The total number of flowers visited by each pollinator taxon was recorded in each census. Further details on methods can be found in Herrera (1995), in which pollinator census data for this population and year were analyzed in a different context.

Population differences in pollinator diversity were studied in 1996 in 15 populations of *L. latifolia* distributed over a broad area of the Sierras de Cazorla and Segura region. The two most distant populations were 55 km apart. Populations included the Arroyo Aguaderillos site studied in 1991 and occurred in pine (*Pinus nigra* or *P. pinaster*) or oak (*Quercus rotundifolia*) woodlands. Pollinators were censused at all populations from 24 July to 14 August. At each site, 80–120 pollinator censuses were conducted on a single date on 20 different *L. latifolia* shrubs, using the same procedures as in 1991 Aguaderillos censuses, except that censuses were of 3-min durations. To investigate annual variation in pollinator diversity and whether population differences remained consistent across years, five of the 15 populations were censused again in 1997 (29 July to 12 August). Pollinators were censused on the same individual plants with the same methods in both years, the only relevant difference being that in 1997 the censuses for each population were spread over several different dates.

Statistical analyses—Individual pollinator censuses are treated as the sampling units in all analyses. The fact that censuses were of different durations in 1991 (5 min) and 1996–1997 (3 min) is inconsequential because censuses of different duration are not mixed in any analysis. The information provided by each census consisted of the list of pollinator species recorded and the total number of flowers visited by each. Censuses without pollinator visits were also included and treated in the same way as quadrats without individuals in conventional species diversity analyses (Colwell, 2000). Treating censuses as sampling quadrats and flower visits as individuals, I computed sample-based rarefaction curves scaled to number of individuals separately for individual shrubs (1991) or populations (1996, 1997), using procedures described by Colwell (2000) and Gotelli and Colwell (2001). As the “individuals” considered here are flower visits rather than biological individuals, I will refer hereafter to “flower-based” rarefaction curves to avoid ambiguity. Plant- or population-specific, average rarefaction curves were obtained with the EstimateS program (Colwell, 2000), using 50 randomizations and sampling without replacement. Rarefaction curves presented in this study depict the expected number of pollinator species represented in a small collection of n flower visits drawn at random from the large pool of N visits. Expected pollinator species richness standardized to a common number of visited flowers obtained from these curves will be used to evaluate the degree of pollinator generalization of individuals and populations.

RESULTS

Within-population variation—A total of 32 pollinator species (13 lepidopterans, 13 hymenopterans, and six dipterans) were recorded in the 437 5-min censuses of the 15 *L. latifolia*

TABLE 1. Sampling effort and pollinator species richness for 15 *Lavandula latifolia* shrubs studied in Arroyo Aguaderillos, Sierra de Cazorla, southeastern Spain, in 1991.

Plant number	Sampling effort		Pollinator species richness	
	Number of censuses	Total flowers visited	S _{OBS}	S _{RAR100} (± 1 SD)
1	29	61	5	—*
2	30	187	4	3.7 ± 0.6
3	30	215	12	6.6 ± 2.0
4	28	43	3	—*
5	29	200	6	3.6 ± 1.2
6	29	481	16	7.1 ± 2.7
7	30	136	5	4.5 ± 0.5
8	29	101	7	7.0 ± 0.2
9	29	432	9	4.1 ± 1.2
10	29	249	10	5.8 ± 1.4
11	29	839	12	2.8 ± 1.5
12	29	66	6	—*
13	29	130	13	11.1 ± 1.6
14	29	259	10	6.0 ± 1.4
15	29	717	16	5.8 ± 2.1

Note: S_{OBS} = observed species richness, the cumulative number of pollinator species recorded during all the censuses on a given plant. S_{RAR100} = rarefaction-estimated species richness, obtained from the flower-based rarefaction curve for each shrub (Fig. 1) as the y-value predicted for $x = 100$ flowers visited. * Non-estimable, because total flowers visited < 100.

shrubs of Arroyo Aguaderillos in 1991. The number of pollinator species recorded per plant (S_{OBS}, Table 1) was much smaller and varied considerably (range 3–16 species per plant; Table 1). Flower visitation also varied widely among plants, ranging from 1.5 ± 4.6 flowers visited/census (mean ± SD) to 28.9 ± 31.6 flowers/census, thus a 20-fold variation ($\chi^2 = 82.6$, $df = 14$, $P \ll 0.001$; Kruskal-Wallis ANOVA). Mean flower visitation and S_{OBS} were positively, significantly correlated across plants ($r = 0.694$, $N = 15$, $P = 0.004$), which suggests that plant differences in S_{OBS} may reflect differential pollinator visitation frequency rather than, or in addition to, true differences in pollinator species richness. Flower-based rarefaction curves computed separately for each plant (Fig. 1) clearly confirm this possibility. The pollinators of some plants with large S_{OBS} values (e.g., plants 6 and 15, S_{OBS} > 15 species) are in fact appreciably less diverse than those of others with lower S_{OBS} (e.g., plants 3, 12, 13, S_{OBS} < 13 species) (Fig. 1).

Flower-based rarefaction curves for individual shrubs (Fig. 1) reveal considerable variation among plants in pollinator species richness. Confidence intervals around each curve have been omitted from the graph to avoid cluttering, but the confidence belts of the most species-poor plants (2, 5, 9 and 11) are largely non-overlapping with those of the most species-rich plants (3, 12, 13). Truly comparable estimates of pollinator species richness were obtained from the rarefaction curves of individual plants as the projected y-value corresponding to an abscissa of $x = 100$ flower visits (S_{RAR100}; Table 1). S_{RAR100} could not be estimated for three plants that were quite infrequently visited by pollinators (total number of flowers visited < 100; Table 1). For the remaining 12 plants, S_{RAR100} ranged from 2.8 to 11.1 species, which denotes that the expected number of pollinator species implicated in the visitation of 100 flowers varied four-fold among shrubs that were < 30 m apart. The correlation between S_{RAR100} and S_{OBS} across plants is not statistically significant ($r = 0.462$, $N = 12$ plants, $P = 0.13$), but becomes highly significant when it is partialled on

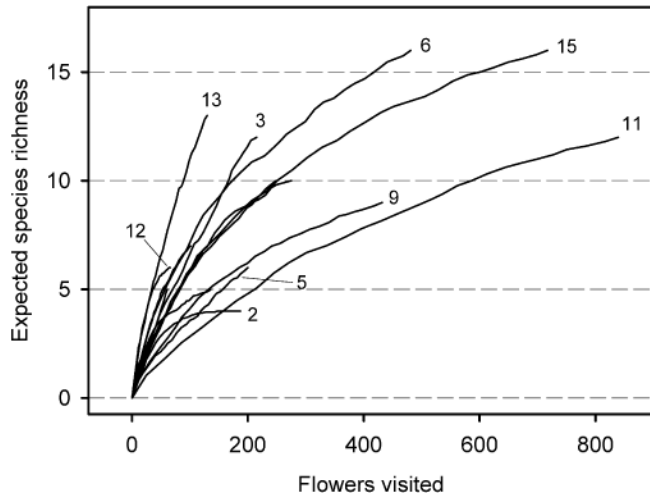


Fig. 1. Flower-based pollinator rarefaction curves depicting the expected accumulation of pollinator species with increasing number of flowers visited, obtained separately for 15 shrubs of *Lavandula latifolia*, Arroyo Aguaderillos, Sierra de Cazorla, southeastern Spain, 1991. Each curve is the average of 50 randomizations without replacement of the censuses conducted on each plant (see Table 1 for census- and flower-based sampling efforts). Numerals identify individual plants referred to in the text.

mean flower visitation per census and the effect of this latter variable is thus statistically accounted for (partial $r = 0.907$, $N = 12$, $P = 0.0001$).

Variation among populations—A total of 60 pollinator species (26 lepidopterans, 23 hymenopterans, and 11 dipterans) were recorded in the 1460 3-min censuses in 1996 at the 15 *L. latifolia* populations. The number of species recorded per locality (S_{OBS} , Table 2) was considerably lower and varied one order of magnitude among sites (range = 3–30 species recorded per site; Table 2). Mean number of flowers visited per 3-min census (\pm SD; all species pooled) varied also considerably among populations, from 0.7 ± 2.9 to 14.2 ± 17.4 flowers/census, thus a 20-fold variation ($\chi^2 = 350.7$, $df = 14$, $P \ll 0.001$; Kruskal-Wallis ANOVA). Across populations, S_{OBS} was directly correlated with mean flower visitation per census ($r = 0.675$, $N = 15$, $P = 0.006$), which suggests that population differences in S_{OBS} largely reflect differences in pollinator visitation. This is supported by flower-based rarefaction curves for the 15 populations studied (Fig. 2), which reveal that S_{OBS} was a poor index of population-level differences in pollinator species richness. For example, the pollinator assemblages of populations 3 and 7, with $S_{OBS} = 30$ and 25 species, respectively, were actually considerably less diverse than that of population 1, with $S_{OBS} = 18$ species.

Flower-based rarefaction curves show that, after accounting for population differences in pollinator visitation frequency, populations of *L. latifolia* still differ widely in pollinator species richness (Fig. 2). Confidence intervals have been omitted from Fig. 2, but those of extreme populations (e.g., populations 1, 3, 7, and 12 vs. 5, 8, 11, and 14) are largely non-overlapping. S_{RAR100} could be estimated for 14 populations, and ranged between 2.9–13.8 species (Table 2). The expected number of pollinator species implicated in the visitation of 100 flowers thus varied nearly five-fold in the set of *L. latifolia* populations studied. The correlation between S_{RAR100} and S_{OBS} across populations was positive and statistically significant (r

TABLE 2. Sampling effort and pollinator species richness estimates for the 15 *Lavandula latifolia* populations studied in the Sierras de Cazorla and Segura, southeastern Spain, in 1996.

Population*	Sampling effort		Pollinator species richness	
	Number of censuses	Total flowers visited	S_{OBS}	$S_{RAR100} (\pm 1 \text{ SD})$
1	100	160	18	13.8 \pm 1.6
2	100	233	6	4.0 \pm 0.9
3	80	1013	30	8.5 \pm 2.0
4	100	318	8	4.6 \pm 0.9
5	80	1133	19	6.4 \pm 1.6
6	100	494	11	5.4 \pm 1.1
7	100	582	25	8.8 \pm 2.6
8	120	148	3	2.9 \pm 0.2
9	100	74	6	—¶
10	120	557	13	3.9 \pm 1.6
11	100	541	9	3.1 \pm 0.8
12	100	660	20	7.5 \pm 1.6
13	100	191	10	6.8 \pm 1.5
14	80	1012	15	5.8 \pm 1.5
15	80	163	11	8.6 \pm 1.3

Note: S_{OBS} = observed species richness, the total number of pollinator species recorded during all the censuses on a particular population. S_{RAR100} = rarefaction-estimated species richness, obtained from the flower-based rarefaction curve for each population (Fig. 2) as the y-value predicted for $x = 100$ flowers visited. * Site names, location (as X-Y UTM coordinates to the nearest km on European 1979 map datum system, UTM zone 30S), and elevation: 1, Arroyo Aguaderillos, 510–4201, 1180 m; 2, Arroyo Amarillo, 505–4193, 1380 m; 3, Arroyo de los Ubios, 508–4199, 1235 m; 4, Caballo de Acero, 514–4195, 1450 m; 5, Collado del Calvario, 510–4200, 1425 m; 6, Cruz de Quique, 504–4194, 1290 m; 7, Cuevas Bermejas, 513–4203, 1210 m; 8, Las Canalejas, 522–4215, 1440 m; 9, Las Navillas, 508–4198, 1170 m; 10, Pista de Los Escalones, 536–4222, 1520 m; 11, Prados de Navahondona, 504–4190, 1540 m; 12, Presilla de Tíscar, 500–4182, 1190 m; 13, Puerto de Tíscar, 497–4183, 1180 m; 14, Raso del Tejar, 511–4203, 1040 m; 15, 250 m SE of Arroyo Aguaderillos, 511–4201, 1210 m.

¶ Non-estimable, because total flowers visited <100.

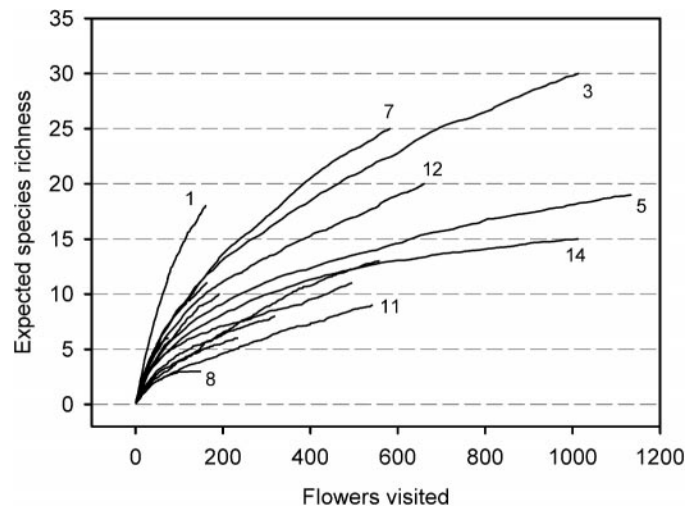


Fig. 2. Flower-based pollinator rarefaction curves showing the expected accumulation of pollinator species with increasing number of flowers visited, obtained separately for 15 populations of *Lavandula latifolia* studied in 1996 in the Sierras de Cazorla and Segura, southeastern Spain. Each curve is the average of 50 randomizations without replacement of the censuses conducted on each population (see Table 2 for census- and flower-based sampling efforts). Numerals identify populations mentioned in the text.

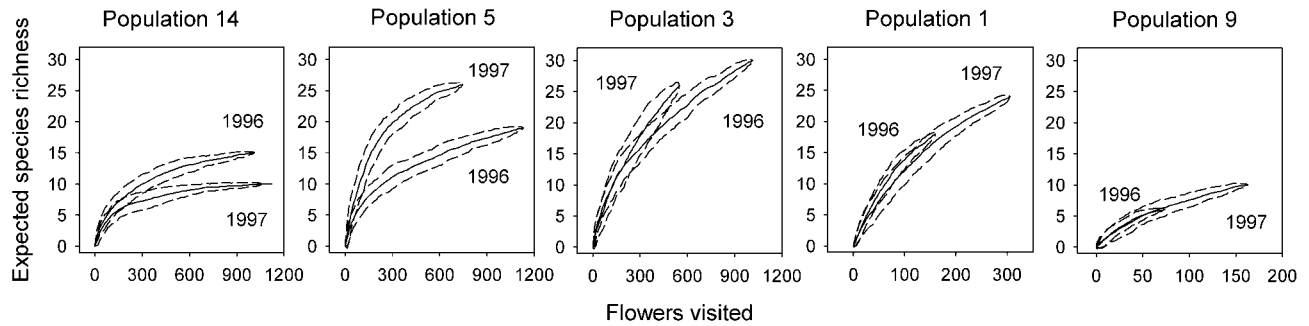


Fig. 3. Flower-based pollinator rarefaction curves for five *L. latifolia* populations studied on two consecutive years. Each curve is an average obtained after 50 randomizations without replacement of the censuses conducted on each population and year. Dashed lines denote ± 1 SD around the mean. Sampling effort for 1996 as shown in Table 2; for 1997, number of censuses and total flowers visited in parentheses: population 1 (65, 306), population 3 (65, 549), population 5 (60, 746), population 9 (60, 163), population 14 (70, 1124). Note different scale of horizontal axis for populations 1 and 9.

= 0.623, $N = 14$ populations, $P = 0.02$), although its magnitude and significance level were enhanced when the influence of pollinator visitation was accounted for by partialing the correlation on mean visitation rate per census (partial $r = 0.783$, $N = 14$, $P = 0.001$).

Between-year comparisons of rarefaction curves for the five populations studied in 1996 and 1997 are shown in Fig. 3. The shape and slope of the curves for the two years were quite similar in three populations (1, 3 and 9). In the other two populations, differences between curves were moderate (population 14) to large (population 5), and the two sites differed in the sign of the change between years. In general, differences among populations in pollinator diversity tended to be larger than differences between years for the same population and, at least in some populations, pollinator diversity seemed a relatively constant, population-level trait.

DISCUSSION

Biases and pitfalls in assessing pollinator diversity—Irrespective of whether they pertain to individual shrubs (Fig. 1) or populations (Fig. 2), the shape of flower-based rarefaction curves obtained in this study was similar to those customarily obtained in investigations of species diversity of ecological communities (Gotelli and Colwell, 2001). Pollinator species are first quickly added as the number of flower visits increases, and the rate of species addition declines progressively as the curve approaches an (expected) asymptote. Similar patterns were obtained when rarefaction curves were scaled to number of censuses rather than number of flowers (graphs not shown). These findings confirm Ollerton and Cranmer's (2002) prediction that the relationship between pollinator diversity and sampling effort should most likely be saturating rather than linear. Consequently, using species/sampling effort ratios to correct for differences in sampling effort among the groups being compared (e.g., species, populations, regions, studies) is probably inappropriate in most instances. Gotelli and Colwell's (2001, pp. 384–385) detailed arguments on the various pitfalls associated with using this kind of “category-subcategory ratios” to compare species diversities thus seem to apply also to estimates of pollinator diversity, and will not be repeated here.

With just a few exceptions (e.g., plant 2 in Fig. 1; populations 8 and 14 in Fig. 2), all pollinator rarefaction curves computed in this study were far from reaching any apparent asymptote. Despite the rather large number of censuses con-

ducted and flower visits recorded, sampling effort was still clearly insufficient to reveal all pollinator species that interact with *L. latifolia* individuals or populations and, therefore, simple counts of the number of pollinator species observed (S_{OBS}) are not valid descriptors of the actual pollinator diversity of either plants or populations. S_{OBS} values for individual plants or populations depart to variable degrees from true species richness set by expected asymptotes because of differences among plants and populations in both species-accumulation slopes and flower-visitation frequencies. This has the important implication that observed variation in S_{OBS} represents a distorted version of actual differences among plants or populations in pollinator diversity. This distortion is clearly illustrated in this study by weak or nonsignificant correlations between S_{OBS} and S_{100RAR} values, and further highlighted by the strong positive correlations found between S_{OBS} and flower visitation rate in both the among-plant and among-population comparisons.

In the present study, sampling effort (number of pollinator censuses) was held relatively constant across groups under comparison. Despite this, however, variation in flower visitation rate alone explained as much as 48% and 45% of among-plant and among-population variance in S_{OBS} , respectively. In the only other study known to me that evaluated the influence of sampling effort on S_{OBS} , Ollerton and Cranmer (2002) found that 36% of variance among plant communities in mean S_{OBS} was accounted for by differences in sampling effort (number of days of observation). Taken together, these figures mean, on one hand, that holding sampling effort constant across groups under comparison is not sufficient to guarantee reliable pollinator diversity estimates. And on the other hand, that raw S_{OBS} figures may ultimately become almost meaningless as descriptors of pollinator diversity when subject to the combined effects of broad variation in both sampling effort and pollinator visitation and neither of these two factors is adequately taken into consideration. This confirms, in the context of pollinator diversity studies, the long-known general principles applying to the measurement of species diversity in ecological communities, that comparing species richness without reference to a taxon sampling curve is problematic at best and that comparing raw taxon counts for two or more assemblages will generally produce misleading results (Gotelli and Colwell, 2001; and references therein). As with conventional species diversity measurements, pollinator taxon sampling curves emerge as the most reliable method to compare pollinator spe-

cies richness among individual plants, populations of the same species, or species.

Recent studies have frequently considered the prevalence of generalized plant–pollinator relationships in nature (e.g., Herrera, 1996; Waser et al., 1996; Olesen, 2000), and analyzed plant–pollinator interaction networks at the local or regional plant community level in relation to hypotheses on pollinator generalization or, more generally, plant adaptation to pollinators (e.g., Memmott, 1999; Dicks et al., 2002; Olesen and Jordano, 2002; Bascompte et al., 2003). These investigations have often relied on plant community-level compilations of pollinator species and/or raw pollinator species counts gathered from preexisting studies (but see, e.g., Memmott, 1999; Dicks et al., 2002; Nakano and Washitani, 2003), have used uncorrected pollinator species counts (S_{OBS}) to measure degree of plant generalization, and have generally paid little or no attention to the potential influence of sampling biases inherent to, and artifacts derived from, using that kind of data (but see Ollerton and Cranmer, 2002). Findings of this study on *L. latifolia* suggest that comparative analyses using raw S_{OBS} values as measures of pollinator diversity are prone to suffer from artifacts caused by heterogeneities in sampling effort, pollinator visitation, or some complex combination of these. This is exemplified by Ollerton and Cranmer's (2002) investigation on latitudinal trends in pollinator specialization. These authors found a significant latitudinal trend in pollinator generalization when raw S_{OBS} values were used, but the pattern vanished when they accounted for differences among sites in sampling effort.

Artifacts derived from neglecting or inadequately accounting for heterogeneities in sampling effort and pollinator visitation frequency may likewise affect in unpredictable ways some community-wide analyses of plant–pollinator networks. In these studies, unaccounted differences among species and communities in sampling effort and/or pollinator visitation may lead to analyzed pollinator spectra being uncorrelated or weakly correlated with actual pollinator spectra across the species or communities involved in the comparison. Furthermore, unappreciated correlations across species or plant communities between apparent pollinator species richness (S_{OBS}) and pollinator visitation frequency are apt to lead to spurious conclusions whereby correlates of pollinator abundance are erroneously interpreted as correlates of pollinator diversity. Disentangling the relative contributions of variations in pollinator abundance and pollinator diversity to observed variation in apparent species richness (S_{OBS}) should become a priority of plant–pollinator community studies. Meanwhile, some conclusions of these investigations are to be treated with caution until their robustness to underlying pollinator sampling inadequacies and hidden abundance–diversity correlations is tested and verified.

Generalization as a local and individual property—At the regional level, the pollination system of *Lavandula latifolia* undoubtedly qualifies as highly generalized. About 85 species of dipteran, hymenopteran, and lepidopteran pollinators were recorded in an earlier investigation conducted in the same area studied here (Herrera, 1988), and a total of 60 pollinator species from the same three major insect groups were recorded in the 15 populations studied in 1996. The present study has shown, however, that extensive generalization is not an invariant, species-level property of *L. latifolia*. Populations vary broadly in degree of pollinator generalization and, within the

highly generalized population of Arroyo Aguaderillos, individual plants are quite variable in their degree of generalization. There, the range of S_{RAR100} values for individual plants in 1991 (2.8–11.1 pollinator species) was strikingly similar to the range of population-level values for the 15 populations studied in 1996 (2.9–13.8 species). This suggests that, after accounting for variation in sampling effort and pollinator visitation, the magnitude of the variation in degree of pollinator generalization occurring at the scale of tens of meters (among shrubs of the same population) may be as large as that occurring at the scale of tens of kilometers (among populations).

An analysis of the correlates of observed variation in degree of pollinator generalization falls beyond the scope of this paper, but available information allows for at least some tentative interpretations. Variation in S_{RAR100} was not significantly related to variation in mean corolla tube length at either the among-population ($r = -0.146$, $N = 14$ populations, $P = 0.62$; C. M. Herrera, unpublished data) or within-population levels ($r = 0.109$, $N = 12$ plants, $P = 0.74$; data in Table 1 and the Appendix in Herrera, 1995). In contrast, location effects seem to account for a significant fraction of observed variation. Pollinator diversity of populations growing adjacent to permanent streams (mean $S_{RAR100} \pm SD = 9.7 \pm 2.8$ species, $N = 4$ populations) was nearly double that of populations on arid slopes (mean $S_{RAR100} = 5.1 \pm 1.8$ species, $N = 10$ populations; $\chi^2 = 6.48$, $df = 1$, $P = 0.01$, Kruskal-Wallis ANOVA; C. M. Herrera, unpublished data). In Aguaderillos in 1991, S_{RAR100} of individual shrubs was inversely correlated with their daily mean solar irradiance ($r = -0.581$, $N = 12$ plants, $P = 0.037$; data in Table 1 and Appendix in Herrera, 1995). Taken together, these relationships suggest that the variable degree of pollinator generalization in populations and individuals of *L. latifolia* may be more parsimoniously explained in terms of abiotic factors influencing insect diversity at the landscape and microsite spatial scales than in terms of variation in some floral trait limiting the range of pollinators. It is not surprising that in the dry, hot summer typical of the Mediterranean-type climate of my study region, *L. latifolia* populations contiguous to the few permanent streams (see also Herrera, 1988) and shrubs that occupy relatively shadier locations in the forest understory have the most diverse insect pollinator assemblages because of the more benign microclimates. In an abiotically driven scenario of this kind, microclimatic factors extrinsic to the plants might be more important than intrinsic plant features in determining the extent of pollinator generalization of *L. latifolia* populations and individuals.

Variation in pollinator composition among populations of the same plant seems to be the rule in nature (e.g., Herrera, 1988; Gómez and Zamora, 1999; Thompson, 2001; Eckert, 2002), thus the finding that populations of *L. latifolia* differed in pollinator diversity was not unexpected. More interesting was the finding that in some populations the shape and slope of pollinator rarefaction curves varied little between years. Although based on rather limited evidence (only five populations studied over two years), this result suggests that some *L. latifolia* populations may interact over the years with pollinator assemblages of a given, locality-specific diversity and that population differences may remain consistent across years. Such a pattern would lead to geographically variable opportunities of adaptation to particular pollinators (Thompson, 1994). The broad variation among populations in extent of pollinator generalization documented in this study (as measured by S_{RAR100}) also lends support to Olesen's (2000) con-

tion that discussions on the evolution of pollinator generalization should focus on the population level. Among-population variability revealed by this study also suggests that single-population data are probably insufficient to characterize a plant species with regard to its degree of generalization. To this end, *both* the central tendency and variability of some suitable population-level estimator of generalization (e.g., S_{RAR100} as used here) obtained at a sufficient number of distinct populations should be used, rather than single figures or pooled averages.

Concluding remarks—This paper has shown that pollinator diversity estimates based on raw species counts may be heavily dependent on aspects related to research design (variation in sampling effort), biological phenomena (differences in pollinator abundance or visitation rates), or both. If unaccounted for, such effects may combine to mask or distort underlying ecological patterns of interest. As shown in this study, flower-based rarefaction curves applied to data obtained through random sampling of pollinator activity at flowers are useful for making rigorous comparisons of pollinator species richness among individual plants, populations of the same species, or different species.

The notion of generalization/specialization gradients has been present in the ecological literature for decades (Futuyma and Moreno, 1988), and the study of factors promoting or limiting specialization has played a central role in the development of entire ecological subdisciplines like, e.g., the study of plant–herbivore interactions (Berenbaum, 1990; Jaenike, 1990). Developments in the study of factors influencing specialization in plant–pollinator systems may likewise catalyze significant improvements in our understanding of the evolution of these mutualistic interactions (Herrera, 1996; Waser et al., 1996; Gómez, 2002). Devising a clearer formalization of concepts and developing more rigorous analytical tools are two prerequisites for such advances to take place. More important than this, however, will be to obtain significant amounts of fresh field data for a broad variety of species, plant communities and ecosystems using adequate sampling protocols allowing for rigorous comparisons and analyses. New methods of analysis cannot compensate for the current scarcity of reliable field data on plant–pollinator interactions, as recently stressed by Kay and Schemske (2004), and sophisticated analytical tools can hardly redeem biased or otherwise messy pollinator data.

Our understanding of the evolution of plant–pollinator interactions will also benefit from a better knowledge of how plant specialization on pollinators varies among individuals of the same population, among distinct populations of the same species and among different species, much in the same way and for the same reasons as progress in the study of plant–herbivore interactions has benefited from recognition of the distinct levels implicated in the evolution of herbivore specialization (Fox and Morrow, 1981; Bernays and Minkenberg, 1997; Bernays and Singer, 2002). As with herbivore specialization on plants, plant specialization on pollinators may be a variable attribute of populations rather than a trait of a species throughout its geographical range and, like in plant–herbivore systems, consideration of pollinator generalization as a local phenomenon will affect the framing of questions about plant–pollinator interactions in both ecological and evolutionary contexts (Fox and Morrow, 1981).

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