

Distribution ecology of pollen tubes: fine-grained, labile spatial mosaics in southern Spanish Lamiaceae

Carlos M. Herrera

Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avenida de Maria Luisa s/n, E-41013 Sevilla, Spain

Summary

Author for correspondence:

Carlos M. Herrera

Tel: +34 95 4232340

Fax: +34 95 4621125

Email: herrera@ebd.csic.es

Received: 10 July 2003

Accepted: 15 September 2003

doi: 10.1046/j.1469-8137.2004.00972.x

- Patterns of intraspecific variation in the number of pollen tubes per style in naturally pollinated plants are poorly known, yet that information is essential for assessing the frequency of occurrence and evolutionary implications of microgametophyte competition in the wild.
- This paper analyses intraspecific variation in the number of pollen tubes per style for six species of southern Spanish insect-pollinated Lamiaceae (*Ballota hirsuta*, *Lavandula latifolia*, *Marrubium supinum*, *Phlomis lychnitis*, *Rosmarinus officinalis* and *Teucrium rotundifolium*) differing in growth form, phenology, flower size and pollinators.
- Number of pollen tubes exceeded number of fertilizable ovules in 26–87% of styles, and mean number per pollen tube of other pollen tubes in the same style varied between five and 12. Within-plant variation in pollen tube number was extensive in all species, accounting for 68–92% of total population-level variance. In *L. latifolia* mean pollen tube number per style differed between populations and between plants within populations, but such differences were not consistent among years.
- It is concluded that opportunities for microgametophyte competition and selection on competitive ability are considerable in all species studied, although extreme spatial fine-graininess and marked stochasticity in the variation of pollen tube numbers, including temporal inconstancy of individual differences, will greatly reduce the opportunity for selection on sporophytic characters influencing degree of microgametophyte competition.

Key words: microgametophyte competition, individual variation, intraspecific variation, Lamiaceae, *Lavandula latifolia*, mean crowding, pollen tube numbers.

© *New Phytologist* (2004) **161**: 473–484

Introduction

Intraspecific variation in the size of pollen loads deposited on stigmas may influence both the number and quality of the eventual progeny. Threshold effects, nonlinear dose–response relationships, and maternal and paternal identity, act in concert to determine the number of seeds produced (Bertin, 1990; Waser & Price, 1991; Holm, 1994; Melser *et al.*, 1997; Mitchell, 1997; Bosch & Waser, 1999; Dieringer & Cabrera, 2002). In addition, the amount of pollen deposited may also influence the quality of the progeny through the action of prefertilization (microgametophyte competition: Snow, 1986; Schlichting *et al.*, 1987;

Winsor *et al.*, 1987, 2000) and/or postfertilization mechanisms (selective abortion: Niesenbaum & Casper, 1994; Rigney, 1995; Havens & Delph, 1996; Niesenbaum, 1999; Melser & Klinkhamer, 2001).

Recognition of the important role played by pollen load size in sexual plant reproduction has motivated a multitude of observational and experimental investigations dissecting, in considerable detail, the patterns and consequences of variation in size of stigmatic pollen loads. This contrasts sharply with the reduced number of investigations that have so far documented patterns of variation in the size of pollen tube populations for naturally pollinated plants in the wild (Levin,

1990; Honig *et al.*, 1992; Aizen & Feinsinger, 1994; Niesenbaum, 1994; Plitmann & Levin, 1996; Herrera J, 1997; Quesada *et al.*, 2001; Herrera CM, 2002). This relative dearth of information is particularly striking, as patterns of natural variation in pollen tube numbers are important for at least the following three reasons. First, pollen load size is just a convenient proxy for the parameter actually influencing seed production and progeny quality, namely the number of pollen tubes that penetrate the stigma and enter the transmitting tissue of the style. It is the size of this microgametophyte population that will actually set an upper limit to the number of fertilizable ovules and, all else being equal, will also determine the possibilities of microgametophyte competition. Given the generally loose relationship existing between the number of conspecific, compatible pollen grains on the stigma and the eventual number of pollen tubes in the style (Snow, 1986; Aizen & Feinsinger, 1994; Herrera J, 1997), the number of pollen tubes that penetrate the stigma is a more proximate variable in the reproductive success of flowers (Plitmann & Levin, 1996). Second, direct measurements of pollen tube number and its relationship to ovule number are essential to assess where individual plants, populations or species stand along the gradient running from extreme pollination deficit (pollen tubes : ovules ratio $\ll 1$) to potentially intense competition between male gametophytes (pollen tubes : ovules ratio $\gg 1$). Documenting patterns of variation in pollen tube numbers within and between species may provide insights on intra- and interspecific patterns of variation in degree of pollen limitation and likelihood of microgametophyte competition. Third, information on the hierarchical apportionment of the variance of number of pollen tubes per pistil between and within individual plants is critical to evaluate the opportunity of selection on sporophytic plant traits influencing the frequency or strength of microgametophyte competition. The fraction of total population-wide variance in pollen tube number accounted for by between-individual differences will set an upper bound to the opportunity of selection on sporophytic characters (e.g. flower or inflorescence traits) potentially influencing degree of pollen tube competition (Herrera, 2002).

Detailed information on the distribution ecology of pollen tube numbers in naturally pollinated populations will therefore allow us to address the following two broad questions: (1) Are conditions conducive to microgametophyte competition sufficiently frequent to generate significant selective forces enhancing microgametophyte competitive ability? (2) Does the spatial (between populations, between plants within populations, within plants) and temporal (between reproductive seasons) structure of variance in pollen tube number allow for consistent selection opportunities on sporophytic characters affecting microgametophyte competition levels? These two questions were addressed by Herrera (2002) in a previous study conducted on Iberian populations of the winter-flowering perennial herb *Helleborus foetidus* L. (Ranunculaceae).

Distribution patterns of pollen tube numbers suggested that, in that species, microgametophyte competition was frequent, yet pollen tube populations were characterized by extremely fine-grained, temporally variable spatial mosaics, and most of total population-wide variance in pollen tube number per pistil was accounted for by variation occurring within individual plants. These earlier findings (see also Levin, 1990; Niesenbaum, 1994; Herrera J, 1997) suggested that, despite microgametophyte competition occurring frequently, there may be little opportunity for selection on sporophytic traits that would influence it (Herrera, 2002). Nevertheless, detailed information on pollen tube populations is still available for very few plants, and further data from a broader spectrum of species are needed before serious generalizations can be made.

This paper describes patterns of spatial and temporal variation in pollen tube numbers in the style of naturally pollinated flowers of six species of southern Spanish insect-pollinated Lamiaceae. The general objective of the study was to address questions (1) and (2) above, in order to test whether the main results of the preceding investigation on *H. foetidus* (Herrera, 2002) are corroborated in this disparate set of species. The specific questions addressed are: (3) Are interspecific differences in growth form, flowering time and pollinators related to variation in the size of pollen tube populations, inferred frequency of microgametophyte competition, or apportionment of variance between and within individual plants? For one of the species studied (*Lavandula latifolia* Medicus), pollen tube data collected over several years from many populations and individuals will be used to address the following additional questions related to intraspecific variation: (4) To what extent do opportunities for microgametophyte competition, and the relative magnitude of within- and between-plant variance in pollen tube number per style, vary between populations?; and (5) Do differences between populations, and between plants of the same population, in mean size of pollen tube populations persist across years?

Materials and Methods

The information presented in this paper was collected during 1996–2002 at 20 different sites located in the Parque Natural de Cazorla-Segura-Las Villas, Jaén province, south-eastern Spain (see Herrera, 2002 for a situation map; Valle *et al.*, 1989 and Luque, 1995 for descriptions of the region's vegetation). Locality names, geographical coordinates and other details of study locations are given in the Results section.

Interspecific patterns

Flower samples of *Ballota hirsuta* Benth., *Lavandula latifolia* Medicus, *Marrubium supinum* L., *Phlomis lychnitis* L., *Rosmarinus officinalis* L. and *Teucrium rotundifolium* Schr. were collected in March–August 2002. These species encompass a broad range of habitat types, growth habits, flower sizes and pollination

Table 1 Growth habit and pollination features of the six species of Lamiaceae studied, referred to populations from the Sierra de Cazorla study region

| Species | Habit | Flowering period | Mean corolla tube length (mm) | Main pollinators |
|-------------------------------------|-------------------------|------------------|-------------------------------|--|
| <i>Ballota hirsuta</i> Benth. | Subshrub | Jun | 9.2 | Bumble bees, honeybees, large Anthophorids |
| <i>Lavandula latifolia</i> Medicus | Shrub | Jul–Sep | 6.9 | Honeybees, Megachilids, Nymphalid butterflies, Syrphid flies |
| <i>Marrubium supinum</i> L. | Suffrutescent perennial | Jun | 7.0 | Bumble bees, small- and medium-sized Anthophorids |
| <i>Phlomis lychnitis</i> L. | Suffruticose perennial | May–Jun | 25.4 | Bumble bees |
| <i>Rosmarinus officinalis</i> L. | Shrub | Mar–Apr | 6.0 | Bumble bees, honeybees, Halictids and Andrenids |
| <i>Teucrium rotundifolium</i> Schr. | Suffrutescent perennial | Jun–Jul | 10.1 | Large Anthophorids |

modes (Table 1). Taken together, their flowering periods extend over nearly 7 months, from March (*Rosmarinus*; each species is designated hereafter by genus name) through September (*Lavandula*). One representative population was sampled per species around the time of local flowering peak or shortly thereafter. Newly withered corollas that had fallen to the ground beneath plants (*Rosmarinus*, *Phlomis*), or dry corollas remaining attached to the calyx after anthesis, were collected on single dates at each population from 20 different plants ($n = 10$ for *Lavandula*) chosen as widely spaced as possible. Collected corollas (10–12 per plant) were stored in vials filled with 2.5 : 2.5 : 95% formaldehyde : acetic acid : ethyl alcohol solution (FAA), separately for different individuals. In all species studied, the style always remains enclosed within withered corollas, and pollen tube counts conducted on this material reflect the final number of pollen tubes in the style at the end of the female stage (coincident with the end of the flower's life, as all species studied are protandrous). It must also be noted that, in the species studied here, persistence of the dry corollas with the enclosed styles was a species-specific trait and that, within species, all corollas either fell shortly after anthesis (*Rosmarinus*, *Phlomis*), or persisted for at least 1–2 wk (the rest of species) after anthesis, irrespective of whether the flower would eventually set fruit or not. This rules out the possibility of the sampling methods used leading to biased data with respect to the number of pollen tubes in styles (a bias of that sort would have been expected if, within a given species, persistence of corollas was contingent on pollination intensity).

Spatio-temporal patterns

Spatio-temporal components of intraspecific variation in pollen tube numbers were studied on *Lavandula* alone. Relevant aspects of the reproductive biology of this species have been described elsewhere (Herrera, 1987a, 1988, 1991, 1995b). In the Sierra de Cazorla region, this low evergreen shrub flowers in July–September. Flowers are hermaphroditic, protandrous, have pale blue tubular corollas, and are produced over a short

(3–6 cm) terminal portion of long-stalked (up to 1.25 m high) inflorescences. Flowers are pollinated by a diverse insect assemblage comprising nearly 80 bee, fly and butterfly species.

Fifteen populations of *Lavandula* were studied in one or more years over the period 1996–2002. They were distributed over a wide area of the Sierra de Cazorla (the two most distant populations were 55 km apart), and encompassed a range of habitat types including pine-dominated (*Pinus nigra* or *P. pinaster*) open woodlands, oak (*Quercus rotundifolia*) forest clearings, and open shrublands where *Lavandula* itself was dominant. In 1996, flowers from the 15 populations were sampled during 24 July–14 August. At each site, corollas from 15–20 newly withered flowers were collected from each of 20 plants, using the same general methods described under 'Interspecific patterns'. Pollen tube data from this large sample ($n = 5533$ flowers, all populations combined) will be used for assessing patterns of intraspecific variation at a regional scale. Annual variation in pollen tube populations, and whether annual changes took place consistently across populations, were investigated on data from a subset of five populations that were sampled again in 1997 (29 July–12 August). The geographical range of these populations was much more restricted than that of the whole 15-site set, the two most distant localities being only 6.2 km apart. The sampling scheme in 1997 involved collecting 20–60 flowers from each of five or six plants in every population ($n = 900$ flowers in total). At each site, individual plants sampled in 1997 were a random subset of those sampled in 1996.

Consistency across years of differences between individual plants in mean number of pollen tubes per style will be examined using data from marked shrubs that were sampled twice: six plants from Arroyo Aguaderillos (population no. 1) and five plants from Raso del Tejar (population no. 14) sampled on 1996 and 1997; and 10 plants from Arroyo Aguaderillos sampled in 2000 and 2002. Unfortunately many of the tags identifying the plants sampled in the extensive 1996 survey could not be found again in 1997, which precluded a more thorough study of interannual consistency of individual variation.

The sampling scheme used in the Arroyo Aguaderillos population in 1997, 2000 and 2002 allowed dissecting within-plant variance in pollen tube numbers into its within- and among-inflorescence components, and assessing whether observed patterns remained consistent among years. Ten individual plants (six in 1997) were sampled there between 25 July and 18 August each year, with four to eight corollas from newly withered flowers being collected separately from each of five to 10 inflorescences per plant.

Laboratory methods

Preserved corollas were dissected in the laboratory to extract the enclosed style. The epifluorescence method of Martin (1959) was used to reveal pollen tubes. Styles were kept at 65°C for 20 min in 0.5 mol l⁻¹ NaOH (1 mol l⁻¹ was used for *Phlomis*) for softening, rinsed in distilled water, and stained for 20 min at 65°C in decolorized aniline blue. The number of pollen tubes penetrating the stigma and reaching the basal third of the stylar canal was then counted under a fluorescence microscope. In two samples (*Teucrium* and *Phlomis*), the number of tubes was counted simultaneously at the basal and distal ends of the style. A close linear relationship between both measurements was found ($r = 0.919$, $n = 228$ for *Teucrium*, $r = 0.810$, $n = 194$ for *Phlomis*). After penetrating the stigma, pollen tube attrition along the style was relatively unimportant in these two species. On average (± 1 SE), $88.3 \pm 2.5\%$ (*Teucrium*) and $80.7 \pm 3.7\%$ (*Phlomis*) of pollen tubes penetrating the stigma eventually reached the basal end of the style. These data, although referred to only two of the six species studied, provide support for using counts of pollen tubes at the basal third of the style as reliable descriptors of the size of pollen tube populations.

Statistical analyses

Statistical analyses were carried out using the SAS statistical package unless otherwise noted. Comparisons between species, population or individual plant means were performed with nonparametric Kruskal–Wallis analysis of variance (K–W ANOVA; Zar, 1984), as implemented in SAS procedure NPAR1WAY. Variance components of pollen tube numbers at the various levels (populations, individuals within populations, inflorescences within plants) were estimated using the restricted maximum likelihood (REML) method, as implemented in procedure MIXED (SAS, 1996). This procedure also provides approximate standard errors of variance component estimates. Procedure MIXED and REML estimation was also used for mixed-model ANOVAs. Interannual consistency in between-population and between-individual differences in mean size of pollen tube populations were assessed by testing the significance of the population \times year and individual \times year interactions, respectively, in two-way ANOVAs. When a significant interaction between main effects occurred, the SLICE option in the LSMEANS statement will be used to assess further

the statistical significance of a given effect at the different levels of the other ('simple main effects'; Schabenberger *et al.*, 2000).

Two complementary measures will be used to evaluate the likelihood of microgametophyte competition. On one side, the proportion of styles with number of pollen tubes > 4 (the number of ovules in the ovary of Lamiaceae) will provide a sporophyte-centred assessment of the frequency of situations where an excess of pollen tubes relative to ovule number will probably result in competition. In addition, a microgametophyte-centred assessment will be obtained using the mean crowding index of Lloyd (1967), m^* , treating styles as 'quadrats' and pollen tubes as 'individuals'. In the context of this study, m^* has a clear biological meaning, as it denotes the mean number per pollen tube of other pollen tubes in the same style. This index provides a quantitative evaluation of the within-style competitive environment faced by individual microgametophytes. Bootstrap estimates of confidence limits for m^* were computed using S-Plus 2000 functions (Mathsoft, 1999) and the bias-corrected and accelerated percentile method (BCa; Efron & Tibshirani, 1993) with 5000 repetitions.

Results

Interspecific patterns

With the exception of *Lavandula*, the species of Lamiaceae studied were remarkably similar in the statistics describing the central tendency and dispersion of the number of pollen tubes per style at the population level (Table 2). Excluding *Lavandula*, means and interquartile ranges were quite similar for all species. There was no significant heterogeneity among species in mean number of pollen tubes per style when data for *Ballota*, *Marrubium*, *Phlomis*, *Rosmarinus* and *Teucrium* are compared ($\chi^2 = 6.14$, $df = 4$, $P = 0.19$; K–W ANOVA). *Lavandula* stood apart from these species in having, on average, about twice as many pollen tubes per style and a largely nonoverlapping interquartile range (Table 2).

The number of pollen tubes per style spanned a broad range in all species, from values considerably lower to values considerably higher than four, the number of ovules in the ovary (Fig. 1). The shape of the frequency distributions differed between species. *Lavandula* and *Marrubium* had approximately normal, unimodal distributions roughly centred around the mean, while distributions for *Ballota*, *Phlomis*, *Rosmarinus* and *Teucrium* were bimodal with one mode around zero and the other around four. The proportion of styles with more than four tubes (range = 26–87%) suggests that situations conducive to microgametophyte competition were quite frequent in flowers of all species (Table 2). Likewise, mean crowding indices (m^* ; range = 4.7–12.3) indicate that, in all species, individual pollen tubes had an average number of neighbour pollen tubes in the same style which exceeded the number of fertilizable ovules.

In all species, individual plants differed significantly in mean size of their pollen tube populations (*Ballota*, $\chi^2 = 73.75$,

Table 2 Summary statistics for number of pollen tubes in styles of six species of Lamiaceae in the Sierra de Cazorla region

| Species ^a | n_p ^b | n_s ^c | Mean \pm 1 SD | Interquartile range | Percentage styles with more than four tubes ^d | Mean crowding ^e |
|-------------------------------|--------------------|--------------------|-----------------|---------------------|--|----------------------------|
| <i>Ballota hirsuta</i> | 20 | 216 | 4.7 \pm 4.2 | 1–7 | 25.9 | 7.4 (6.6–8.7) |
| <i>Lavandula latifolia</i> | 10 | 319 | 10.7 \pm 5.2 | 7–14 | 86.8 | 12.3 (11.7–13.0) |
| <i>Marrubium supinum</i> | 20 | 175 | 4.6 \pm 2.3 | 3–6 | 43.5 | 4.7 (4.3–5.2) |
| <i>Phlomis lychnitis</i> | 20 | 218 | 5.4 \pm 4.2 | 2–8 | 42.7 | 7.6 (6.7–8.7) |
| <i>Rosmarinus officinalis</i> | 20 | 203 | 4.9 \pm 4.3 | 2–7 | 30.5 | 7.7 (6.5–9.8) |
| <i>Teucrium rotundifolium</i> | 22 | 228 | 4.7 \pm 3.9 | 2–7 | 32.0 | 6.9 (6.1–7.9) |

^aCollection site, location (as X–Y coordinates to the nearest km on European 1950 datum system, UTM zone 30S), elevation, and sample collection date: *Ballota hirsuta*, Cerrada del Utrero, 507–4198, 950 m, 25 June; *Lavandula latifolia*, Arroyo Aguaderillos, 510–4201, 1180 m, 17 August; *Marrubium supinum*, Nava del Espino, 509–4195, 1430 m, 26 June; *Phlomis lychnitis*, near Vadillo, 506–4198, 1020 m, 24 June; *Rosmarinus officinalis*, Raso del Tejar 1030 m, 26 March; *Teucrium rotundifolium*, Nava Cabeza del Tejo, 511–4196, 1650 m, 18 August.

^b n_p = Number of individual plants sampled. ^c n_s = Number of styles examined. ^dLamiaceae flowers have a constant number of four ovules per ovary. ^eLloyd (1967) mean crowding index, m^* . In parentheses, 95% confidence intervals of estimates determined by bootstrapping.

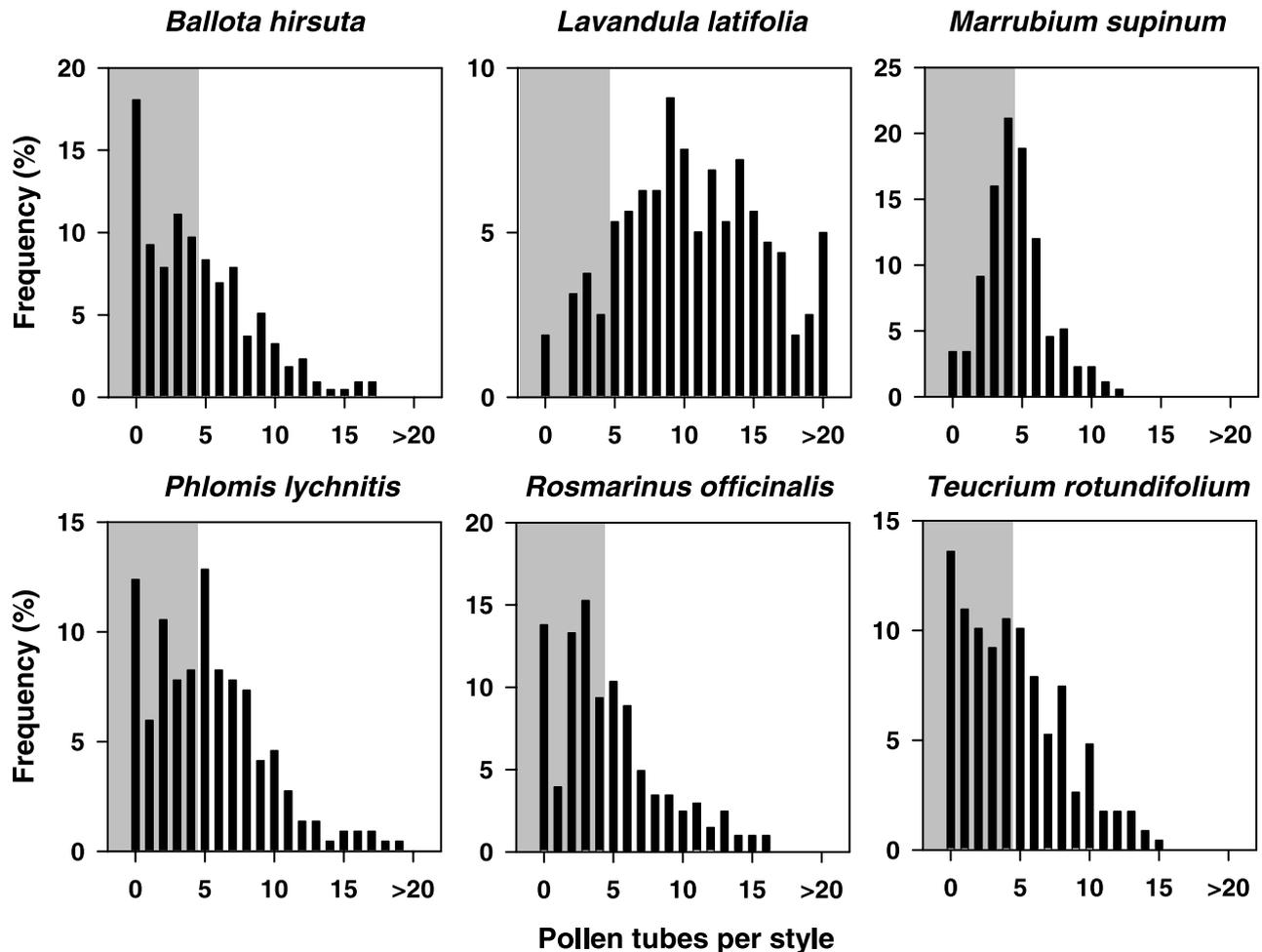


Fig. 1 Frequency distributions of the number of pollen tubes per style in six species of Lamiaceae of the Sierra de Cazorla region, south-eastern Spain. A single population was sampled for each species in 2002. In each graph, shaded portion denotes area of distribution where the number of pollen tubes is ≤ 4 , the number of ovules in the ovary. See Table 2 for sampling locations, sample sizes and summary statistics.

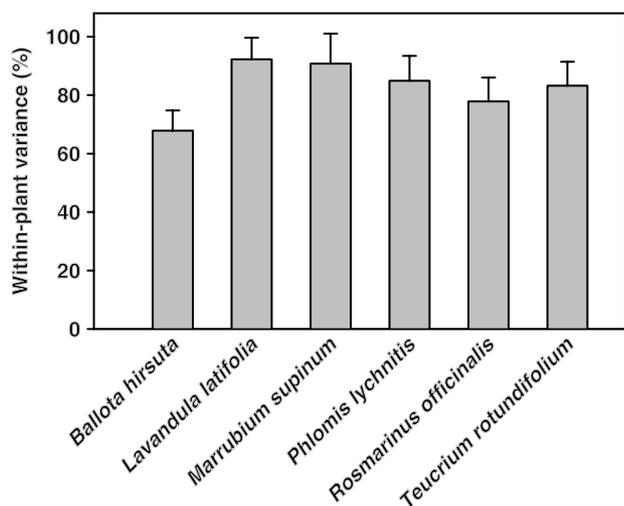


Fig. 2 Proportion of population-level variance in number of pollen tubes per style accounted for by within-plant variation (among flowers of the same individual) for the six species of Lamiaceae studied. Vertical segments denote 1 SE of estimates. Based on the absolute variance component estimates (± 1 SE) shown in parentheses after species names (among- and within-plant components, respectively): *Ballota hirsuta* (5.76 ± 2.24 , 12.17 ± 1.23); *Lavandula latifolia* (2.13 ± 1.39 , 25.41 ± 2.04); *Marrubium supinum* (0.49 ± 0.34 , 4.84 ± 0.55); *Phlomis lychnitis* (2.65 ± 1.31 , 14.93 ± 1.50); *Rosmarinus officinalis* (4.13 ± 1.79 , 14.52 ± 1.52); *Teucrium rotundifolium* (2.52 ± 1.15 , 12.51 ± 1.23).

$df = 19$, $P < 0.0001$; *Lavandula*, $\chi^2 = 29.80$, $df = 9$, $P = 0.0005$; *Marrubium*, $\chi^2 = 30.80$, $df = 19$, $P = 0.042$; *Phlomis*, $\chi^2 = 50.54$, $df = 19$, $P < 0.0001$; *Rosmarinus*, $\chi^2 = 49.68$, $df = 19$, $P < 0.0001$; *Teucrium*, $\chi^2 = 58.91$, $df = 21$, $P < 0.0001$; K–W ANOVA). In quantitative terms, however, variation between plant means was always relatively minor in comparison with variation between the flowers of the same plant, as illustrated by the results of partitioning total intraspecific, population-level variance into its between- and within-plant components (Fig. 2). The within-plant component of variance ranged between 67.9% (*Ballota*) and 92.3% (*Lavandula*) of total, and it was >80% in three other species (*Marrubium*, *Phlomis*, *Teucrium*). A variance partitioning conducted on the data of the six species combined revealed that variation between species (23.2% of total variance) and between individuals within species (11.8% of total) in number of pollen tubes per style was relatively unimportant in comparison to variation within plants (65.0% of total). On average, therefore, the variance in pollen tube numbers between flowers of the same plant nearly doubled the variance between species and conspecific individuals combined.

Variation at population and individual levels

The 15 populations of *Lavandula* sampled in 1996 were significantly heterogeneous in mean number of pollen tubes per style ($\chi^2 = 466.6$, $df = 14$, $P < 0.0001$; K–W ANOVA), population means ranging between 8.3 and 15.8 tubes/style (Table 3). No

significant relationship was found between this variable and either population elevation ($r_s = 0.259$, $P = 0.35$) or contiguity to permanent streams ($\chi^2 = 0.07$, $df = 1$, $P = 0.79$; K–W ANOVA), a variable known to influence pollinator composition and abundance in this species (Herrera, 1988). No significant relationship was likewise found between the matrix of dissimilarity between populations (constructed using pairwise differences in mean pollen tubes per style) and the matrix of geographical distances ($r = 0.044$, $P = 0.75$; Mantel permutation test with 1000 repetitions). Both the proportion of styles with more than four tubes (range = 52–89%) and the mean crowding index (range = 12–18) varied widely between populations (Table 3), and the high values found indicate ample opportunities for microgametophyte competition at all *Lavandula* populations studied.

Significant heterogeneity among individual *Lavandula* plants in mean number of pollen tubes per style was the rule in the populations studied. Differences between plant means were highly significant ($P < 0.0005$ or better) in 12 populations, and marginally significant in the other three ($0.04 < P < 0.07$). Despite statistical significance, however, differences between plant means were generally negligible in comparison with variation occurring between flowers of the same plant. Separate partitions of variance conducted for the different populations reveal that as much as 76–99% of population-wide variance in pollen tube numbers (mean \pm SD = $85.6 \pm 7.6\%$) was accounted for by within-plant variation (Table 3). The overwhelming importance of the within-plant variance is further highlighted by partitioning the total variance in pollen tube numbers for the 15 populations combined. Variance between populations accounted for 13.5% (SE = 1.5), variance between plants within populations for 8.4% (SE = 3.5), and variance between flowers of the same plant accounted for 78.0% (SE = 1.5) of the total regional variance in pollen tube numbers at the broad spatial scale encompassed by the 15 populations.

The flower sampling scheme adopted in 1997, 2000 and 2002 at the Aguaderillos population involved collecting flowers from five to 10 different, widely spaced inflorescences within each plant. This allowed for further partitioning the within-plant variance component in pollen tube numbers into within- and among-inflorescence components, and also for assessing the relative magnitude of these components in relation to between-plant variance. Results are summarized in Table 4. In the three study years, nearly all the population-wide variance in pollen tube numbers occurring in the Aguaderillos population was accounted for by differences between flowers of the same inflorescence (87–93% of total). In neither year was there any detectable variance component attributable to differences between inflorescences of the same plant.

Annual variation

Five of the *Lavandula* populations studied in 1996 were sampled again in 1997. The effects of year, population, and their interaction on the number of pollen tubes were tested with a two-way

Table 3 Summary statistics for number of pollen tubes in styles of *Lavandula latifolia* in 15 populations of the Sierra de Cazorla region

| Population number ^a | n_s^b | Mean \pm 1 SD | Interquartile range | Percentage styles with more than four tubes | Mean crowding ^c | Within-plant variance (%) (SE) ^d |
|--------------------------------|---------|-----------------|---------------------|---|----------------------------|---|
| 9 | 344 | 8.3 \pm 6.1 | 3–13 | 53.8 | 11.7 (11.0–12.3) | 96.2 (7.6) |
| 1 | 384 | 8.9 \pm 7.1 | 3–14 | 51.8 | 13.5 (12.7–14.4) | 75.7 (5.6) |
| 11 | 287 | 9.5 \pm 8.0 | 3–14 | 55.8 | 15.1 (13.7–16.7) | 81.5 (7.0) |
| 13 | 393 | 9.9 \pm 7.7 | 3–16 | 52.7 | 14.9 (14.1–15.8) | 83.0 (6.1) |
| 7 | 381 | 9.9 \pm 6.2 | 5–14 | 76.4 | 12.8 (12.0–13.7) | 89.7 (6.7) |
| 5 | 399 | 10.2 \pm 6.2 | 6–14 | 74.6 | 12.9 (12.2–13.6) | 88.5 (6.4) |
| 8 | 407 | 10.9 \pm 7.3 | 5–16 | 69.7 | 14.8 (14.0–15.7) | 96.9 (7.0) |
| 4 | 398 | 11.4 \pm 7.4 | 5–16 | 72.1 | 15.3 (14.5–16.2) | 82.5 (6.0) |
| 14 | 390 | 11.4 \pm 7.7 | 5–18 | 66.6 | 15.7 (14.9–16.5) | 77.0 (5.7) |
| 15 | 312 | 11.8 \pm 7.0 | 7–16 | 80.1 | 15.0 (14.0–16.0) | 83.0 (6.9) |
| 6 | 375 | 12.1 \pm 6.8 | 7–17 | 78.1 | 14.9 (14.1–15.7) | 75.7 (5.7) |
| 2 | 419 | 13.7 \pm 7.2 | 8–19 | 84.8 | 16.5 (15.8–17.1) | 86.5 (6.1) |
| 3 | 267 | 14.7 \pm 7.2 | 10–19 | 89.1 | 17.1 (16.3–18.1) | 89.7 (8.0) |
| 12 | 376 | 15.0 \pm 7.9 | 9–20 | 87.5 | 18.2 (17.3–19.0) | 79.2 (5.9) |
| 10 | 401 | 15.8 \pm 7.5 | 10–21 | 89.1 | 18.4 (17.7–19.1) | 98.7 (7.2) |

Populations listed in increasing order of means to facilitate comparisons. ^aSite names, location (as X–Y coordinates to the nearest km on European 1950 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 Tiscar, 500–4182, 1190 m; 13, Puerto de Tiscar, 497–4183, 1180 m; 14, Raso del Tejar, 511–4203, 1040 m; 15, 250 m SE of Arroyo Aguaderillos, 511–4201, 1210 m. ^b n_s = Number of styles examined at each site ($n = 20$ plants per population). ^cLloyd (1967) mean crowding index, m^* . In parentheses, 95% confidence intervals of estimates, determined by bootstrapping. ^dPercentage of total population-wide variance in number of pollen tubes per style accounted for by variation among flowers on the same plant.

Table 4 Partition of total population-wide variance in number of pollen tubes per style for the *Lavandula latifolia* Aguaderillos population in three different years

| Year | n_p^a | n_s^b | Total population variance | Percentage variance (SE) | | |
|-------------------|---------|---------|---------------------------|--------------------------|----------------------------|-----------------------------|
| | | | | Between plants | Within plant | |
| | | | | | Inflorescence within plant | Flower within inflorescence |
| 1997 | 6 | 177 | 46.77 | 13.0 (10.1) | 0 | 87.0 (9.4) |
| 2000 ^c | 10 | 488 | 43.96 | 7.4 (4.3) | 0 | 92.6 (6.0) |
| 2002 ^c | 10 | 319 | 27.54 | 7.7 (5.0) | 0 | 92.3 (7.4) |

^a n_p = Number of plants sampled. ^b n_s = number of styles examined. In each plant, flowers were sampled separately from five to 10 different inflorescences. ^cThe same plants sampled in the two years.

ANOVA, with plant identity incorporated as a random effect. The effects of both population ($F_{4,116} = 3.73$, $P = 0.007$) and year ($F_{1,116} = 12.16$, $P = 0.0007$) were statistically significant, but these results cannot be interpreted separately because of the significant population–year interaction ($F_{4,116} = 4.64$, $P = 0.0016$). Population means did not vary in unison from year to year in the five populations and, as a consequence, between-population differences did not remain consistent between years (Fig. 3). The mean number of pollen tubes per style increased from 1996 to 1997 in four populations, and decreased in one population. The rank of population means in the two years were uncorrelated ($r_s = -0.1$, $P = 0.94$), and the population

with the largest average pollen tube populations in 1996 (population 3) became the one with the smallest population in 1997. Differences between populations were statistically significant in 1996 ($F_{4,116} = 12.50$, $P < 0.0001$), but not in 1997 ($F_{4,116} = 1.87$, $P = 0.12$; tests of simple main effects).

Consistency of individual variation

Using data from individual *Lavandula* shrubs that were sampled in different years, this section examines whether the differences between plants at a given population remained consistent across years. Three data sets will be considered: (I) five plants from

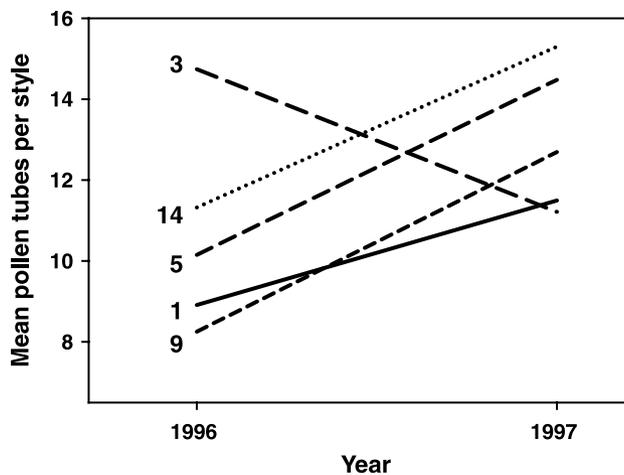


Fig. 3 Annual variation in mean number of pollen tubes per style in five populations of *Lavandula latifolia* sampled in 1996 and 1997. Each line corresponds to a different population, identified by their codes in Table 3. Values plotted are the model-adjusted, least-squares means resulting from a two-way ANOVA with year and population as main fixed effects, and plants as random effects (see text for statistical significance of effects). Sample sizes for 1996 are shown in Table 3; for 1997, $n = 338, 136, 152, 93$ and 181 flowers for populations 1, 3, 5, 9 and 14, respectively.

Raso del Tejar (population no. 14); (II) six plants from Arroyo Aguaderillos, each sampled in 1996 and 1997; and (III) 10 plants from Arroyo Aguaderillos, each sampled in 2000 and 2002. The effects of individual, year and their interaction on mean number of pollen tubes per style were tested separately on each data set using two-way ANOVAs. As the individual and year main effects have already been considered, this section focuses on the individual–year interaction, which informs on the degree of consistency of individual differences across years.

The individual–year interaction was highly significant in data sets I ($F_{4,256} = 22.60, P < 0.0001$) and II ($F_{5,435} = 7.32, P < 0.0001$), and marginally significant in data set III ($F_{9,787} = 1.78, P < 0.068$). In these populations and years, therefore, individual differences in mean number of pollen tubes per

style did not remain consistent across years or, in other words, annual variation in mean number of pollen tubes per style at the population level did not affect all individual plants similarly (Fig. 4). Tests of simple main effects indicate that, in each population, individual means changed significantly from one year to another in some individuals (four out of five plants in data set I; three out of six plants in data set II; six out of 10 plants in data set III), but not in others. As a result, the ranking of individuals with respect to mean pollen tube number changed drastically from one year to another. This was particularly marked in data sets I and II (Fig. 4), where the ranking of individuals was reversed from 1996 to 1997, as denoted by the significant ($r_s = -1.000, P < 0.001$; data set I) or nearly significant ($r_s = -0.754, P = 0.08$) negative rank correlation coefficients. Plants with the densest pollen tube populations in 1996 thus turned into those having the sparsest ones in 1997, and *vice versa* (Fig. 4).

Discussion

Interspecific patterns

The six species of Lamiaceae considered in this study differ widely in growth form, flowering season, inflorescence architecture, flower size and pollinators. In addition, they occupy a variety of habitat types, including dense Mediterranean-type sclerophyllous shrublands (*Rosmarinus*), sparse low scrub on xeric slopes (*Phlomis*, *Lavandula*), crevices in limestone cliffs (*Teucrium*), meadows in open pine woodlands (*Marrubium*), and nitrified soil patches amidst boulders beneath high cliffs (*Ballota*). The finding of this study that a group of species otherwise so diverse are similar in most descriptive parameters of their pollen tube populations contrasts with initial expectations based on the species' disparity. Most species were remarkably similar in statistics describing the central tendency and dispersion of pollen tube numbers per style. Except for *Lavandula*, species means and ranges of variation of pollen tube numbers were almost identical in all species, whose means did not differ significantly. Furthermore, species were also quite similar in

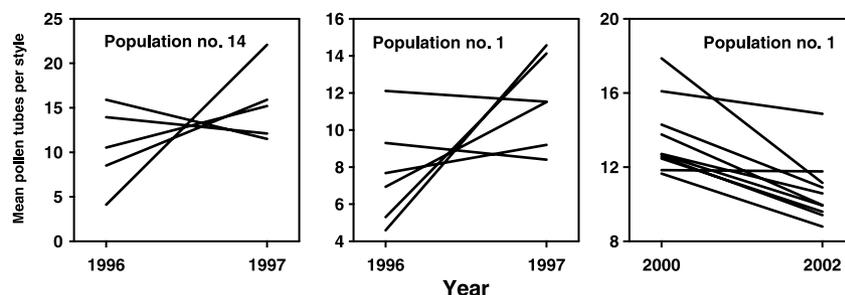


Fig. 4 Annual variation in mean number of pollen tubes per style for individual *Lavandula latifolia* plants of populations 1 and 14 which were sampled in two different years. In each graph segments connect mean values of the same shrub in two different years. Means plotted are model-adjusted, least-squares means resulting from separate two-way ANOVAs with plant and year as main effects (see text for statistical significance of the corresponding plant–year interaction effects). In population 1, different individuals were sampled in 1996–97 and 2000–02.

exhibiting considerable opportunities for microgametophyte competition, as judged from both the sporophytic (proportion of styles with more than four pollen tubes) and gametophytic (mean crowding index) perspectives. *Lavandula* stood apart as the species where opportunities for microgametophyte competition were greatest, with 87% of styles having more than four tubes and a mean crowding index = 12 (each pollen tube had an average of 12 neighbour tubes in the same style).

In all species except *Lavandula*, pollen tube means were roughly equal to the number of ovules per ovary, and average pollen tube : ovule ratios (PT : O ratio hereafter) fell in a narrow range close to unity (1.15–1.35). The PT : O ratios of these species are inferior to values previously reported in the literature for other naturally pollinated plants, which ranged between 1.5 and 5.2 (reviewed by Herrera, 2002), and are only comparable to the low values obtained in the same study region for the winter-flowering *H. foetidus* (PT : O = 0.95; Herrera, 2002). The values found in this study for the summer-flowering *Lavandula* (PT : O = 2.68) are higher, and fall within the interval reported in the literature for other plants. These results suggest a seasonal trend in my study region, with PT : O ratios increasing from winter- to summer-flowering species. Assuming that mean PT : O provides a rough indication of the opportunities of microgametophyte competition, such a seasonal trend would entail a parallel increase in the likelihood of microgametophyte competition, and a decline in the frequency of pollen limitation, from winter- through summer-flowering plants. This hypothesis needs to be substantiated by additional investigations on more species, but is consistent with results of earlier studies in the region, which have consistently found weak pollination deficits in three winter-flowering species (*Narcissus longispathus*, *H. foetidus*, *Daphne laureola*; Herrera, 1995a, 2002; Herrera *et al.*, 2001; C. Alonso, personal observation), but not in the summer-flowering *Lavandula* (Herrera, 1991).

Within-plant variation

All species studied exhibited extensive within-plant variation in pollen tube numbers, which was considerably greater than variation between individual plants. For species considered individually, the within-plant component accounted for between 68 and 92% of total population-level variance in pollen tube numbers. For all species combined, the within-plant component nearly doubled the variance as a result of interspecific and individual variation combined. Furthermore, the 15 *Lavandula* populations studied in 1996 were similar in the overwhelming importance of the within-plant variance component (range 76–99%) and, for all populations combined, variation between flowers of the same plant was responsible for 78% of total regional variance. In the single *Lavandula* population that was sampled in three different years, the importance of the within-plant variance component remained consistently high and fluctuated within very narrow limits (87–93%) across years.

There are few comparable investigations providing data on patterns of variation of pollen tube numbers in populations of naturally pollinated plants. Within-plant variation was also a prominent source of variation in pollen tube numbers in *Lindera benzoin* (85% of total regional variance; Niesenbaum, 1994); *H. foetidus* (approx. 50%; Herrera, 2002); and *Helleborus viridis* (60–85%; J. Guitián and C.M.H., personal observations). The studies of Levin (1990) on *Phlox drummondii* and of Honig *et al.* (1992) on *Staberoha banksii*, although they did not present formal variance partitioning analyses, also showed as much or more variation among pistils of single plants as among plants. These earlier results, along with those presented here for six species of Lamiaceae, provide compelling evidence supporting the notion that extensive within-plant variation is probably the rule in naturally pollinated populations in the wild.

The large within-plant component of variation in pollen tube numbers appears to be associated with very fine-grained variation within plants. Results shown here for *Lavandula* indicate that, in that species, within-plant variation was almost entirely caused by differences between flowers in the same inflorescence, which are only 3–6 cm apart. The variance component caused by differences between inflorescences of the same plant (30–50 cm apart) was nonestimable, and thus assumed to be equal to zero, in the three study years. In *H. foetidus*, flowers of which are apocarpous, about half the within-plant variance in pollen tube numbers was caused by differences among styles of the same flower, which are only a few millimetres away from each other (Herrera, 2002). In *H. viridis* (also an apocarpous species), 40–80% of the within-plant variance is accounted for by differences between styles of the same flower (J. Guitián and C.M.H., personal observation). These results suggest that the within-plant component of variation in pollen tube numbers probably arises because of stochastic variations over very short distances in the number, vigour and/or donor diversity of pollen grains reaching the stigma. Small-scale variations in pollen germinating behaviour and performance due, for instance, to variable degrees of clumping, paternal composition, or deposition patterns (Thomson, 1989; Holm, 1994; Németh & Smith-Huerta, 2002) may also contribute to such small-scale stochasticity.

Spatial and temporal patterns in *Lavandula*

The 15 populations of *Lavandula* sampled in 1996 differed significantly in mean pollen tube numbers per style, with extreme population means encompassing a twofold range, from eight up to 16 tubes/styles. This regional variation, however, was neither distance-dependent nor predictably related to variation in the two potentially influential environmental features considered, namely site elevation and contiguity to permanent streams. In *Lavandula*, abundance and taxonomic diversity of pollinators are greatest at populations growing close to permanent streams, and decrease at populations on arid slopes (Herrera, 1988). In the winter-flowering *H. foetidus*, mean size

of pollen tube populations was found to decline with increasing elevation (Herrera, 2002). Differences between *Lavandula* populations in elevation and proximity to streams might thus have contributed to explaining their differences in pollen tube numbers, via effects on pollinator composition and abundance, but neither variable did. Other environmental parameters not considered in this study (e.g. population differences in habitat-specific patterns of solar irradiance on the forest floor; Herrera, 1995b) might have influenced pollinator composition and could thus perhaps have contributed to population differences. Some results of this study, however, tend to suggest that regional variation in the pollen tube populations of *Lavandula* reflects an underlying fine-grained, inconstant spatial mosaic whose characteristics vary between flowering seasons at both among-population and within-population (among individual plants) spatial scales.

Two indirect lines of evidence support this latter interpretation. First, there was a significant population–year interaction effect on mean pollen tube numbers per style for the subset of five *Lavandula* populations sampled in 1996 and 1997. This interaction reflected a decoupling of supra-annual patterns of variation of the different populations. In this respect, it must be noted that the five populations sampled in 1996 and 1997 were distributed over an area of only a few kilometres. Despite their close proximity, differences between populations did not remain consistent over two consecutive flowering seasons, and population means differed significantly in one year (1996) but not in the other. The second line of evidence supporting the view of a very fine-grained, temporally unstable spatial mosaic in pollen tube numbers in *Lavandula* comes from consideration of annual variation in individual plant means. Within populations, differences in plant means did not remain consistent across years, as revealed by the statistically significant individual–year interactions. Within sites, the ranking of individual plants with respect to mean pollen tube numbers varied drastically from year to year. This means that the shrub-centred mosaic in pollen tube numbers occurring at the restricted spatial scale of local populations was also temporally labile and inconsistent between flowering seasons.

The proximate mechanism(s) responsible for the temporal instability of the spatial mosaic at both within and between population levels may be tentatively suggested. Pollinators of *Lavandula* differ in frequency of pollen deposition on the stigma, number of pollen grains delivered per effective pollinating visit, and relative frequency of geitonogamous vs cross-pollinations (Herrera, 1987a; 1987b). The composition of pollinators varies between years and populations (Herrera, 1988). Annual fluctuations of the main pollinators are asynchronous across populations (C.M.H., personal observation), which could easily give rise to the observed population–year interaction on pollen tube numbers. Within populations, differences between *Lavandula* shrubs in pollinator composition are mainly caused by differential location of plants on the forest floor irradiance mosaic (Herrera, 1995b). Even though the irradiance mosaic

and the location of plants will not change between years, annual variations in local pollinator composition, in combination with the differential response of species to the irradiance mosaic (Herrera CM, 1997), might still originate a plant–year interaction effect on pollen tube numbers, as found here.

Fine-grained mosaics and microgametophyte competition

The significance of fine-graininess and temporal inconstancy in the spatial structure of variance of pollen tube numbers in relation to the evolutionary significance of microgametophyte competition in natural populations has been discussed by Herrera (2002) in the context of results obtained for *H. foetidus*. The present study was undertaken to corroborate those patterns in a group of species differing greatly from *H. foetidus* in pollination ecology and taxonomic affiliation. The results presented here corroborate the earlier results for *H. foetidus*, and document a situation even more extreme with respect to levels of within-plant variation and spatio-temporal inconsistency in pollen tube populations. In addition to the temporal inconsistency of differences among populations already documented for *H. foetidus*, this study has shown for the first time that, within local populations, individual differences in the size of pollen tube populations are inconsistent over years, which suggests a further, particularly relevant element of stochasticity. Conclusions tentatively advanced by Herrera (2002) are thus reinforced by the present study on six species of Lamiaceae. First, opportunities for microgametophyte competition and selection on competitive ability are considerable in all species, yet its likelihood and intensity are expected to vary greatly between years, regions, populations, individual plants, and flowers of the same plant. Second, the overt predominance of a within-plant, very small-scale, highly stochastic component of variance in pollen tube numbers will greatly reduce the opportunity for selection on sporophytic characters (e.g. flower or inflorescence traits) influencing degree of pollen tube competition. Third, even if local pollen donors differ in pollen tube growth rate and seed-siring ability, and their differences are heritable and remain consistent across maternal parents (Marshall, 1998; Skogsmyr & Lankinen, 2000, 2002), marked temporal stochasticity among maternal plants in the mean competitive environment within styles will probably lead to (1) reduction in opportunities for selection among pollen donors based on differential pollen performance; and (2) 'protection' from the eroding action of natural selection of additive genetic variation underlying differences in male competitive ability. Additional information on the distribution ecology of pollen tubes from other species and habitat types is needed to ascertain the possible generality of these conclusions.

Acknowledgements

C. Alonso, M. C. Anstett, A. J. Manzaneda, D. Ramírez, R. Requerey and A. Tíscar assisted with *Lavandula* field collections.

The Consejería de Medio Ambiente, Junta de Andalucía, authorized my research in Cazorla and made available invaluable facilities. Mark Rausher and one anonymous reviewer provided very helpful comments on an earlier version, and the former also suggested using Lloyd's mean crowding index to evaluate the competitive environment faced by pollen tubes. The research was funded by grants PB96-0856 from Ministerio de Educación y Cultura, and BOS2000-1122-C03-01 from Ministerio de Ciencia y Tecnología. This paper is gratefully dedicated to Rocío Requerey, who carried out all the laboratory work and tirelessly counted the $\approx 10^5$ pollen tubes which form the basis for this study.

References

- Aizen MA, Feinsinger P. 1994. Forest fragmentation, pollination, and plant reproduction in a chaco dry forest, Argentina. *Ecology* 75: 330–351.
- Bertin RI. 1990. Effects of pollination intensity in *Campsis radicans*. *American Journal of Botany* 77: 178–187.
- Bosch M, Waser NM. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *American Journal of Botany* 86: 871–879.
- Dieringer G, Cabrera L. 2002. The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). *American Journal of Botany* 89: 991–997.
- Efron B, Tibshirani RJ. 1993. *An introduction to the bootstrap*. New York, NY, USA: Chapman & Hall.
- Havens K, Delph LF. 1996. Differential seed maturation uncouples fertilization and siring success in *Oenothera organensis* (Onagraceae). *Heredity* 76: 623–632.
- Herrera CM. 1987a. Components of pollinator 'quality': comparative analysis of a diverse insect assemblage. *Oikos* 50: 79–90.
- Herrera CM. 1987b. Componentes del flujo génico en *Lavandula latifolia* Medicus: polinización y dispersión de semillas. *Anales Jardín Botánico de Madrid* 44: 49–61.
- Herrera CM. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* 35: 95–125.
- Herrera CM. 1991. Dissecting factors responsible for individual variation in plant fecundity. *Ecology* 72: 1436–1448.
- Herrera CM. 1995a. Floral biology, microclimate, and pollination by ectothermic bees in an early-blooming herb. *Ecology* 76: 218–228.
- Herrera CM. 1995b. Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* 76: 1516–1524.
- Herrera CM. 1997. Thermal biology and foraging responses of insect pollinators to the forest floor irradiance mosaic. *Oikos* 78: 601–611.
- Herrera CM. 2002. Censusing natural microgametophyte populations: variable spatial mosaics and extreme fine-graininess in winter-flowering *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* 89: 1570–1578.
- Herrera CM, Sánchez-Lafuente AM, Medrano M, Guitián J, Cerdá X, Rey PJ. 2001. Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* 88: 1025–1032.
- Herrera J. 1997. The role of colored accessory bracts in the reproductive biology of *Lavandula stoechas*. *Ecology* 78: 494–504.
- Holm SO. 1994. Pollination density – effects on pollen germination and pollen tube growth in *Betula pubescens* Ehrh. in northern Sweden. *New Phytologist* 126: 541–547.
- Honig MA, Linder HP, Bond WJ. 1992. Efficacy of wind pollination: pollen load size and natural microgametophyte populations in wind-pollinated *Staberoha banksii* (Restionaceae). *American Journal of Botany* 79: 443–448.
- Levin DA. 1990. Sizes of natural microgametophyte populations in pistils of *Phlox drummondii*. *American Journal of Botany* 77: 356–363.
- Lloyd M. 1967. Mean crowding. *Journal of Animal Ecology* 36: 1–30.
- Luque P. 1995. *Mapa de Vegetación del Parque Natural de Las Sierras de Cazorla, Segura y Las Villas*. Sevilla, Spain: Agencia de Medio Ambiente, Junta de Andalucía.
- Marshall DL. 1998. Pollen donor performance can be consistent across maternal plants in wild radish (*Raphanus sativus*, Brassicaceae): a necessary condition for the action of sexual selection. *American Journal of Botany* 85: 1389–1397.
- Martin FW. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* 34: 125–128.
- Mathsoft. 1999. *S-Plus 2000 Guide to Statistics*, 2. Seattle, WA, USA: Mathsoft.
- Melser C, Klinkhamer PGL. 2001. Selective seed abortion increases offspring survival in *Cynoglossum officinale* (Boraginaceae). *American Journal of Botany* 88: 1033–1040.
- Melser C, Rademaker MCJ, Klinkhamer PGL. 1997. Selection on pollen donors by *Echium vulgare* (Boraginaceae). *Sexual Plant Reproduction* 10: 305–312.
- Mitchell RJ. 1997. Effects of pollination intensity on *Lesquerella fendleri* seed set: variation among plants. *Oecologia* 109: 382–388.
- Németh MB, Smith-Huerta NL. 2002. Effects of pollen load composition and deposition pattern on pollen performance in *Clarkia unguiculata* (Onagraceae). *International Journal of Plant Sciences* 163: 795–802.
- Niesenbaum RA. 1994. Spatial and temporal variation in pollen tube numbers in *Lindera benzoin* (spicebush). *Canadian Journal of Botany* 72: 268–271.
- Niesenbaum RA. 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). *American Journal of Botany* 86: 261–268.
- Niesenbaum RA, Casper BB. 1994. Pollen tube numbers and selective fruit maturation in *Lindera benzoin*. *American Naturalist* 144: 184–191.
- Plitmann U, Levin DA. 1996. Microgametophytes in flowers with and without fruits of *Phlox drummondii* (Polemoniaceae). *Plant Systematics and Evolution* 201: 211–221.
- Quesada M, Fuchs EJ, Lobo JA. 2001. Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree *Pachira quinata* (Bombacaceae). *American Journal of Botany* 88: 2113–2118.
- Rigney LP. 1995. Postfertilization causes of differential success of pollen donors in *Erythronium grandiflorum* (Liliaceae): nonrandom ovule abortion. *American Journal of Botany* 82: 578–584.
- SAS. 1996. *SAS/STAT Software: Changes and Enhancements through Release 6.11*. Cary, NC, USA: SAS Institute.
- Schabenberger O, Gregoire TG, Kong F. 2000. Collections of simple effects and their relationship to main effects and interactions in factorials. *American Statistician* 54: 210–214.
- Schlichting CD, Stephenson AG, Davis LE, Winsor JA. 1987. Pollen competition and offspring variance. *Evolutionary Trends in Plants* 1: 35–39.
- Skogsmyr I, Lankinen Å. 2000. Potential selection for female choice in *Viola tricolor*. *Evolutionary Ecology Research* 2: 965–979.
- Skogsmyr I, Lankinen Å. 2002. Sexual selection: an evolutionary force in plants? *Biology Reviews* 77: 537–562.
- Snow AA. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. *American Journal of Botany* 73: 139–151.
- Thomson JD. 1989. Germination schedules of pollen grains: implications for pollen selection. *Evolution* 43: 220–223.

Valle F, Gómez-Mercado F, Mota Poveda JF, Díaz de la Guardia C. 1989. *Parque Natural de Cazorla, Segura y Las Villas. Guía Botánico-Ecológica*. Madrid, Spain: Editorial Rueda.

Waser NM, Price MV. 1991. Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. *Ecology* 72: 171–179.

Winsor JA, Davis LE, Stephenson AG. 1987. The relationship between

pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. *American Naturalist* 129: 643–656.

Winsor JA, Peretz S, Stephenson AG. 2000. Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). *American Journal of Botany* 87: 527–532.

Zar JH. 1984. *Biostatistical analysis, 2nd edn*. Englewood Cliffs, NJ, USA: Prentice Hall.



About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at **www.newphytologist.org**
- Regular papers, Letters, Research reviews, Rapid reports and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – average first decisions are just 5–6 weeks. Essential colour costs are **free**, and we provide 25 offprints as well as a PDF (i.e. an electronic version) for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £108 in Europe/\$193 in the USA & Canada for the online edition (click on 'Subscribe' at the website)
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 592918) or, for a local contact in North America, the USA Office (newphytol@ornl.gov; tel 865 576 5261)