

Reprinted from:

L. D. Harder and S. C. H. Barrett, editors. *Ecology and evolution of flowers*. Oxford Univ. Press, 2006.

CHAPTER 15

Geographical context of floral evolution: towards an improved research programme in floral diversification

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Outline

The diversification of animal-pollinated angiosperms is related to divergence in floral characteristics promoted by adaptations to different pollinators. According to prevailing evolutionary theory, this macroevolutionary pattern results from adaptive local or regional differentiation of pollination-related features in response to spatial divergence in pollinators. This crucial process links the micro- and macroevolution of floral adaptation, yet it has received much less attention than either floral diversification of species in a phylogenetic context, or pollinator-mediated phenotypic selection on pollination-related traits within populations. This chapter includes two components. We first use a literature survey to demonstrate that the study of plant–pollinator interaction in a geographical context is a relatively neglected element of research on floral diversification. In addition, the few studies that explicitly assess intraspecific variation in pollinators and pollination-related traits generally do not provide unequivocal evidence for a *causal* role of divergent selection from pollinators in intraspecific differentiation in floral traits. We then describe an analysis of regional variation in pollinators and corolla traits (upper lip and corolla tube length) of *Lavandula latifolia*, a Mediterranean evergreen shrub, which illustrates a five-step protocol for identifying geographical differentiation in floral traits driven by spatially variable selection from pollinators. Corolla traits, pollinator composition, and phenotypic selection on the upper corolla lip all vary geographically, and the morphological and pollination-related selection clines are closely congruent. Our results for this species implicate adaptive intraspecific floral differentiation in response to a cline in pollinator-mediated selection on pollination success, although confirmation of this conclusion awaits experiments to determine the genetic basis of floral variation.

15.1 Introduction

Since we view transpecific evolution as an extension of events at the species level, the foundation of most evolutionary theory rests upon inferences drawn from geographic variation or upon the verification of predictions made about it. Gould and Johnston (1972, p. 457)

The causal role played by animal pollinators in the extraordinary diversification of angiosperm

flowers has figured prominently in plant biology since Darwin. The connection between floral diversity and divergence in pollination mechanisms of animal-pollinated lineages was recognized early in the history of evolutionary biology (Darwin 1862; Leppik 1957; Stebbins 1970). Several lines of evidence implicate animal pollinators in angiosperm diversification, including the fact that taxonomically distinctive traits primarily

involve reproductive characters for animal-pollinated lineages, but not for abiotically pollinated taxa (Grant 1949); the temporal match in geological time between the radiations of angiosperms and major groups of animal pollinators (Grimaldi 1999); the frequent association between suites of floral traits and particular pollinator groups (Fenster *et al.* 2004); evidence of more rapid and/or extensive diversification in lineages of animal-pollinated plants (Eriksson and Bremer 1992; Ricklefs and Renner 1994; Dodd *et al.* 1999); and phylogenetic analyses showing that floral form has played a key role in the speciation of some animal-pollinated lineages (Graham and Barrett 2004; Sargent 2004; Chapter 17).

Recently, research on the adaptive origin of floral diversity in animal-pollinated angiosperms has generally adopted one of two approaches. On the one hand, and largely as a consequence of the increased availability of molecular phylogenies, a growing number of investigations have examined the ecological and pollination correlates of floral diversification in a phylogenetic context at the species level and above (Hapeman and Inoue 1997; Graham and Barrett 2004; Patterson and Givnish 2004; Chapter 17). On the other hand, many studies have assessed pollinator-mediated phenotypic selection on floral traits within populations by measuring the fitness consequences of floral variation that occurs naturally (Campbell *et al.* 1991; Herrera 1993; Maad 2000; Chapter 14) or has been induced artificially (Herrera 2001; Aigner 2004; Castellanos *et al.* 2004). The profusion of investigations adopting these approaches contrasts with the scarcity of studies of floral diversification that focus on intraspecific floral variation and its relation to geographic divergence in pollinators.

As summarized in Gould and Johnston's (1972) statement quoted at the beginning of this chapter, the hypothesis that macroevolutionary patterns represent the aggregate outcomes of microevolutionary processes at the intraspecific level is a central tenet of current evolutionary thought (Simpson 1953; Bock 1970). Local adaptation to contrasting pollination environments is an important component of adaptive floral diversification (e.g., Dilley *et al.* 2000; Patterson and Givnish 2004; Chapter 16). For this reason, studies of intraspecific

geographical differentiation in floral traits and its potential relation to divergent selection from pollinators are crucial for understanding the linkage between the micro- and macroevolution of floral traits. Similar arguments have been raised by Barrett (1995; Barrett *et al.* 2001) in relation to the evolution of plant mating systems. However, despite their interest and significance, relatively few studies have addressed the relation of intraspecific floral differentiation to geographically changing selection from pollinators, and most of these do not make convincing cases for pollinator-driven intraspecific differentiation, as discussed below.

In this chapter, we address the geographical context of floral evolution with a literature overview and a detailed, stepwise analysis of a case example. We begin by reviewing the relevant literature from two perspectives. First, we demonstrate that research on floral diversification has largely neglected the geographical context of plant-pollinator interactions. Then, we consider published evidence of intraspecific geographical differentiation in floral form and function and its relation to variation in pollinator faunas, highlighting some limitations that commonly hinder adaptive interpretations of observed patterns. Finally, we outline a relatively simple, stepwise protocol for identifying instances of geographical differentiation in floral traits driven by spatially variable selection from pollinators. We illustrate this approach with a study of geographical variation in the flowers and pollinators of *Lavandula latifolia*, a Mediterranean, evergreen shrub.

15.2 Representation of geographical variation in pollination studies

The neglect of geographical context by studies of floral diversification is evident from the remarkable scarcity of well-documented cases of pollinator-driven intraspecific geographical differentiation in floral form or function in recent books or reviews dealing with local differentiation in plants (Linhart and Grant 1996), ecological speciation (Levin 2000), or the geographical mosaic theory of plant-animal coevolution (Thompson 1994). To quantify this subjective impression, we

conducted two literature surveys as described in the following two sections. First, we reviewed the literature looking for descriptions of geographic variation in floral characteristics and their pollinators. Next, we searched for studies that went beyond patterns and quantified processes, specifically, phenotypic selection on pollination-related plant traits. In both cases we were interested in evaluating the frequency of studies that considered geographical variation.

15.2.1 Patterns: how much attention has geographical variation in plant traits and pollinators received?

We screened the primary literature for papers describing both a plant species' pollinator fauna and one or more floral or plant traits putatively related to pollination. These studies were classified according to whether they provided data on geographical variation. Floral traits could be functional (e.g., dichogamy, floral longevity, nectar secretion rate) or structural (e.g., floral morphology, nectar composition, inflorescence height). We considered only studies conducted under natural field conditions, excluding studies performed in a glasshouse or in experimental plots or arrays, or that involved manipulated plant traits. The survey comprised articles published from 1995 until June 2005 that were accessible to us online; the starting year was later than 1995 for five journals with limited online availability. The journals screened and the first year reviewed (if different from 1995) were: *American Journal of Botany*, *Annals of Botany*, *Canadian Journal of Botany* (1998), *Ecography* (2000), *Ecological Monographs*, *Ecology*, *Evolution*, *International Journal of Plant Sciences*, *Journal of Evolutionary Biology*, *Oecologia* (1997), *Oikos* (2000), and *Plant Systematics and Evolution* (2001). These publications represent major outlets for pollination studies and thus likely provide a representative sample of published research in this field. We initially queried the ISI Web of Science database with the string "pollinator or pollination biology or pollinated" for each journal. The resulting articles ($N=867$) were examined individually if the abstract indicated suitable content. Two reviewers performed the searches and classified the studies,

one examining odd years and the other even years, to reduce possible biases.

Studies were classified according to whether they studied geographical variation in pollinator composition, abundance or visitation rates, and whether they studied geographical variation in plant traits (Table 15.1). By "geographical variation" we mean examination of at least two populations of the same plant species. We included plant species individually in the table, so that multi-species studies contributed more than one species. The upper-left cell in Table 15.1 includes single-site studies that reported only *quantitative* measures of plants and pollinators. This group excludes investigations that measured plant traits but mentioned only the main pollinators, and studies that quantified pollinator composition but provided simple descriptions of floral features. In contrast, for the upper-right and lower-left cells we relaxed the requirement that both plant traits and pollinator composition be measured quantitatively, because very few papers described variation in either plants or pollinators among sites, but quantified the other aspect in only one site. Also, because we were interested in studies that considered geographical aspects, we wanted to ensure that they all were included in the table. As a result of this procedure, the number of studies in the upper-left cell might be underestimated, but this conservative approach reinforces the conclusions drawn below. Finally, the lower-right cell in

Table 15.1 The incidence with which pollination-biology studies published during 1995–2005 in 12 ecological and botanical journals (see text for details) considered geographical variation in pollinator composition and pollination-related plant traits.

Sites studied for pollination-related plant traits	Sites studied for pollinator composition	
	1	> 1
1	525 (79.1)	27 (4.1)
> 1	62 (9.3)	50 (7.5)

Numbers in each cell represent the number of species considered, with the percentage of the overall total in parentheses. A list of the literature references used to construct this table is available upon request or in Electronic Appendix 15.1 (<http://www.eeb.utoronto.ca/EEF/>).

Table 15.1 includes studies that quantified both pollinator composition and pollination-related plant traits for more than one locality. Many of these papers did not compare localities (i.e., they were not testing for geographical variation explicitly), yet we adopted the conservative procedure of including them if findings for different populations were reported separately.

The final survey (Table 15.1) included 198 articles, which provided both pollinator and floral data for 664 plant species. The vast majority of species included in our sample provided information about pollinators and/or pollination-related traits for only one population. For only 7.5% of species were data on pollinator composition and pollination-related traits reported for multiple populations. Information on geographical variation was provided for an additional 13.4% of species, but it referred to either pollinators or plant traits alone, with information on plant traits being twice as common as that for pollinators. These results illustrate unequivocally that pollination biologists rarely consider the geographical context, even though our threshold for a study to qualify for “geographical variation” was quite liberal (number of populations > 1). Almost no studies would have been characterized as considering geographic variation if we had applied a slightly more restrictive threshold (e.g., number of populations > 3).

15.2.2 Processes: how much do we know about geographical variation in selection on pollination-related traits?

Our second literature survey considered studies of phenotypic selection (*sensu* Lande and Arnold 1983) on floral and other pollination-related traits. To make this search as comprehensive as possible, we did not limit the journals or years examined. We used a combination of sources to locate studies, including citations in review articles (e.g., Kingsolver *et al.* 2001) and searches of the ISI Web of Science. To be included, studies had to be conducted under natural pollination conditions and measure phenotypic selection on some character(s) hypothesized by the author(s) to be under pollinator-mediated selection. Glasshouse or flight cage

studies were not considered. Selection had to be measured on traits with typical variation: artificially induced trait variation was acceptable only if it was kept within the range of phenotypic variation for the species. We included studies on both discrete (e.g., flower colour) and continuous (e.g., corolla size) traits. These criteria excluded studies using artificial conditions (e.g., controlled pollinator identity or extreme floral variation) to study phenotypic selection on plant traits, but we were more interested in studies of selection in the wild than in research designed to explore the mechanisms of selection. Likewise, we may have missed some studies of selection on modified floral or plant traits, because they often do not describe their results as “phenotypic selection.” Because experimental studies are not generally replicated geographically, their exclusion should not bias our conclusions.

Results of our survey of phenotypic selection studies are summarized in Table 15.2, which includes data from 62 publications and 66 plant species. For only 39% of these species did the studies examine the possibility of geographical variation in selection by comparing phenotypic selection gradients among populations. However, despite this relative scarcity, the proportion of geographically informed studies was somewhat higher in this case than among the studies of general pollination biology surveyed in the preceding section (Table 15.1). This difference may

Table 15.2 Characteristics of published studies of phenotypic selection on pollination-related plant traits.

Type of pollination-related traits	Is phenotypic selection compared among populations?	
	No	Yes
Structural	25 (64.1)	14 (35.9)
Functional	5 (62.5)	3 (37.5)
Both trait types	10 (52.6)	9 (47.4)
Total	40 (60.6)	26 (39.4)

Numbers in each cell represent the number of studied species, with the corresponding percentages of the row total in parentheses. A list of the literature references used to construct this table is available upon request or in Electronic Appendix 15.1 (<http://www.eeb.utoronto.ca/EEF/>).

indicate that researchers who go beyond description of pollinators and plant traits to investigate the fitness consequences of floral variation under a particular pollination regime are more often aware of the importance of documenting variation in selective regimes among populations. However, this interpretation is contradicted by the fact that only 6 of the 26 geographically informed studies summarized in Table 15.2 quantified population differences in pollinators along with differences in phenotypic selection on plant traits. Therefore, phenotypic selection studies are not an exception to the predominant neglect of a geographical context in investigations of pollinator-mediated floral evolution.

15.3 Outcomes and limitations of geographically informed studies

15.3.1 Outcomes

This section summarizes the outcomes of the few studies in the preceding literature surveys that measured geographical variation in both plant traits and their pollinators (50 species from Table 15.1 plus 6 species from Table 15.2). We asked two questions for this subset of studies: (1) how often did pollinators *and* plant traits vary significantly among populations of the same species; and (2) when both plant traits and pollinators varied significantly, how often was the observed floral variation consistent with patterns expected from adaptive intraspecific diversification mediated by pollinators. To this end, we examined in detail studies in the lower-right cell of Table 15.1, and those in Table 15.2 that included information on pollinators, classifying them according to whether significant inter-population variation was found in floral traits, pollinator composition, or both. Populations were compared for only 33 species, and the outcomes of these studies are summarized in Table 15.3.

Plant-pollinator systems commonly vary geographically: 60.6% of the species included in Table 15.3 exhibit joint geographical variation in plant traits and pollinators. Many investigations published in journals or years not covered by our surveys also confirm the widespread occurrence of simultaneous geographical variation

Table 15.3 The incidence of significant geographical variation in pollinator faunas and pollination-related plant traits, based on the studies referred to in Tables 15.1 and 15.2.

Significant geographical variation in pollination-related plant traits?	Significant geographical variation in pollinators?	
	No	Yes
No	5 (15.1)	3 (9.1)
Yes	5 (15.1)	20 (60.6)

Numbers in each cell represent the number of species, with the percentage of the overall total in parentheses. A list of the literature references used to construct this table is available upon request or in Electronic Appendix 15.1 (<http://www.eeb.utoronto.ca/EEF/>).

in pollination-related traits and pollinator composition (e.g., Miller 1981; Armbruster 1985; Arroyo and Dafni 1995; Inoue *et al.* 1996; Boyd 2002; Malo and Baonza 2002). Studies of 13 of the 33 species included in Table 15.3 explicitly considered the association of floral variation or phenotypic selection on floral traits with variable pollinator faunas. In other words, less than half of these investigations were designed to assess whether geographical variation in floral traits was congruent with pollinator variation. Eight studies of seven species presented compelling evidence for congruent variation between plant traits and pollinator composition (Johnson and Steiner 1997; Gómez and Zamora 1999, 2000; Fausto *et al.* 2001; Totland 2001; Blionis and Vokou 2002; Elle and Carney 2003; Valiente-Banuet *et al.* 2004).

15.3.2 Limitations and a proposal

Except for two cases (see below), most studies included in Table 15.3 claiming that variation in pollinator faunas explained observed patterns of geographical variation in floral traits (or its lack thereof) relied entirely on correlative evidence. These studies described parallel spatial variation of floral traits and one or several aspects of the pollinator assemblage (e.g., taxonomic composition, abundance, mean body size) that may affect selection on the variable floral characters. In some cases, the correlative evidence for pollinator-driven intraspecific diversification is compelling. For

instance, Valiente-Banuet *et al.* (2004) related variation in time of anthesis across the geographic range of a columnar cactus to the variable availability of bat pollinators. In areas where bats are migratory, flowers remain open and secrete nectar during the day, allowing diurnal and nocturnal visitors, whereas flowers are exclusively nocturnal where bats visit reliably. Correlative evidence has also been used to argue for uncoupled geographical variation between plant traits and pollinators, as in Herrera *et al.*'s (2002) study on variation of floral integration in the perennial herb, *Helleborus foetidus*, over the Iberian Peninsula. In our literature review, only studies by Gómez and Zamora (2000) and Totland (2001) assessed variation in plant traits and pollinators in conjunction with geographical variation in phenotypic selection.

If intraspecific variation reflects local adaptation, morphology or function should associate with those aspects of the environment that influence natural selection (e.g., Gould and Johnston 1972). However, the opposite need not be true, and character–environment correlations do not demonstrate a causal relation. Correlations linking geographical variation in flower traits with variation in pollinators of the sort often used, for example, to document “pollination ecotypes” (e.g., Robertson and Wyatt 1990; Arroyo and Dafni 1995; Johnson 1997) suggest only a plausible role of pollinators as agents of floral diversification. Floral traits could vary geographically for three reasons. First, floral traits could exhibit phenotypic plasticity in response to spatially variable environments. In this case the environmental factor(s) inducing floral variation (e.g., flower size) may also cause pollinator variation (e.g., species composition, mean body size). Second, floral variation among populations could reflect neutral phenotypic variation arising from genetic drift. In this scenario, floral variation would cause pollinator differences by “filtering out” available pollinators via, for example, morphological matching or differential exclusion, so that pollinator differences between populations are a proximate ecological consequence, rather than the ultimate evolutionary cause, of floral variation (i.e., an “ecological fitting” scenario *sensu* Janzen 1985). Finally, floral traits could vary geographically in response to

divergent natural selection. Unequivocal demonstration of this process requires additional information on the crucial mechanism that differentiates it from the other two possible processes, namely evidence of spatially variable, pollinator-mediated selection on the floral traits involved. Therefore, in this respect studies of intraspecific floral adaptation conducted in a geographical context are no exception to the established principle that environment–trait correlations are the weakest and least conclusive evidence of natural selection (Lewontin 1974; Endler 1986).

Geographically informed studies of pollinator-driven intraspecific floral differentiation can be strengthened most simply by incorporating an explicit analysis of spatially heterogeneous selection. A study's ability to differentiate between phenotypic plasticity, neutral phenotypic variation and divergent natural selection, and thus reliably identify possible instances of pollinator-driven intraspecific diversification, will be enhanced considerably by the following five-step approach. Step 1 involves the usual practice of documenting geographical variation in pollinators. It must be stressed that, to allow for reliable geographical comparisons, pollinator composition studies should pay careful attention to sampling issues, as discussed in detail by Ollerton and Cranmer (2002) and Herrera (2005), for example. Step 2 tests whether geographically variable floral traits are subject to selection from pollinators. Step 3 examines whether the selection gradient on the floral traits is related to geographic variation in the pollinator fauna. Step 4 quantifies the spatial correlation between variable selection gradients and phenotypic values. Finally, step 5 determines whether population differences in floral traits have a genetic basis. Step 3 is the key component in this protocol. It represents an extended version of the “pollinator \times floral-character interaction” approach suggested by Wilson and Thomson (1996) to account for pollinator-mediated floral divergence. It is also related to the ANCOVA-based phenotypic selection models proposed by Strauss *et al.* (2005) to test for differences in diffuse selection exerted on plants by different species groups of animals (see also Wade and Kalisz 1990). We will apply this five-step protocol in the following section to the study of

clinal variation of *Lavandula latifolia* flowers and their pollinators.

15.4 A case study: clinal variation of *Lavandula latifolia* flowers and pollinators

Lavandula latifolia is a summer-flowering, insect-pollinated shrub of open woodlands in southern France and the eastern Iberian Peninsula (Fig. 15.1a). Flowers are hermaphroditic and self-compatible, but < 4% of flowers set fruit in the absence of pollinators. More than 100 species of bees, flies and butterflies pollinate *L. latifolia* in southeastern Spain, so this species is an outstanding example of generalist pollination at the regional level (Herrera 1988, 2005). Below, we focus mainly on geographical variation in Hymenoptera and Lepidoptera, the two main groups of pollinators, whose proportions vary widely among *L. latifolia* populations. On average, Hymenoptera and Lepidoptera visitors differ in components of pollinating effectiveness, including flower visitation rate, frequency of pollen deposition on the stigmas, mean number of pollen grains left when deposition occurs, and the proportion of interfloral flights between flowers

on different plants (Herrera 1987, 1989). Artificially induced variation in the relative abundance of major pollinator groups affects variable seedling recruitment prospects on a per-flower basis (Herrera 2000). Hymenoptera and Lepidoptera differ in morphology, foraging behaviour, thermal biology, and nutritional requirements, which presumably cause contrasting flower preferences and selection patterns. Therefore, the *L. latifolia*–Hymenoptera–Lepidoptera pollination system is characterized regionally by a combination of (1) non-equivalence of main pollinators in their potential fitness consequences for the plants; (2) possible differences among the main pollinators in flower selection; and (3) variation among populations in pollinator composition (Herrera 1988). This combination provides a suitable background for investigating the possibility of pollinator-driven geographical differentiation in pollination-related floral traits.

15.4.1 Methods

Floral form, pollinator composition, and the maternal component of pollination success, were studied concurrently during July–August 1996 on 300 *L. latifolia* plants from 15 widely spaced

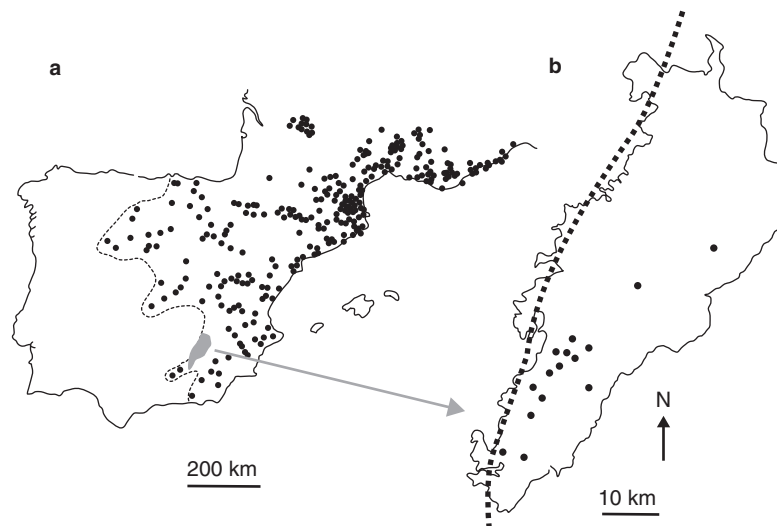


Figure 15.1 The distribution of (a) *Lavandula latifolia* on the European side of the western Mediterranean region (data from Upson and Andrews [2004] and Proyecto Anthos [<http://www.programanthos.org>]) and (b) the 15 populations of *L. latifolia* in Cazorla-Segura-Las Villas Natural Park considered in this chapter (dots). The dotted lines depict the western range limit of *L. latifolia*.

populations in the Sierras de Cazorla and Segura, around the southwestern limit of the species' range (Fig. 15.1b). Pollinator observations were repeated during 1997 in five populations. Twenty shrubs were marked at each site, and pollinators were observed on them between 0730 and 1230 h GMT. Four to six 3-min pollinator censuses were conducted on each plant (sample sizes shown in Electronic Appendix 15.2, <http://www.eeb.utoronto.ca/EEF/>). All flower visitors were identified to species and the number of flowers visited was recorded. Further details on pollinator observation methods are given by Herrera (2005). At each site, 20–25 open flowers were collected from each shrub during the afternoon of the corresponding pollinator census and stored in formaldehyde–acetic acid–ethyl alcohol solution. Flowers last for only 1.5–2.5 days and wither shortly after pollination (Herrera 2001, and unpublished), so pollen grains on the stigmas of afternoon-collected flowers could be related confidently to the activity of pollinators recorded during the preceding morning. For each flower, the lengths of the upper corolla lip and corolla tube (UL and CT hereafter, respectively; Fig. 15.2) were measured under a dissecting microscope using an ocular micrometer, and the numbers of pollen grains on the stigma and pollen tubes in the style were counted under an epifluorescence microscope (Herrera 2004).

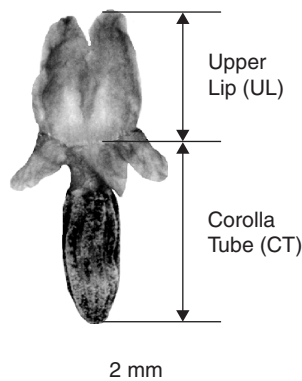


Figure 15.2 *Lavandula latifolia* flower in front view, showing the two measurements used to characterize floral morphology and symbols used in the text.

15.4.2. Step 1: Characterize geographical variation in pollinators

A total of 60 pollinator species (26 Lepidoptera, 23 Hymenoptera, and 11 Diptera) were recorded during the 1460 3-min observation periods at the 15 *L. latifolia* populations studied. The identity of the locally most important species of pollinators varied considerably among sites. Up to ten different taxa ranked among the two most important local pollinators at one site or another (Electronic Appendix 15.2): *Anthidiellum breviusculum* was one of the top two pollinators at nine sites, *Apis mellifera* at eight sites, *Macroglossum stellatarum* at four sites, *Bombus pascuorum* at three sites, *Bombus terrestris* at two sites, and *Ceratina* spp., *Anthophora quadrifasciata*, *Megachile pilidens* and *Lasioglossum* spp. at one site each. Only six of the 15 sites shared the same pair of top-two species (*Apis mellifera* plus *Anthidiellum breviusculum*).

Populations differed broadly in the relative contributions of Hymenoptera, Lepidoptera and Diptera to total floral visits (Fig. 15.3). Hymenoptera were the only or predominant (>80% of flower visits) visitors in six populations, Lepidoptera predominated in one population, and a variable mixture of Hymenoptera, Lepidoptera and Diptera occurred at the remaining seven sites. Diptera had minor importance in all sites and are not considered hereafter. Within populations, the relative occurrence of Lepidoptera tended to decline, and that of Hymenoptera to increase, from south to north ($r=0.514$, $N=15$, $P<0.05$ for Hymenoptera; $r=-0.477$, $N=15$, $P<0.10$ for Lepidoptera; correlations between latitude and population-level importance figures). This latitudinal trend is also evident for visits per plant (Fig. 15.4). Population differences in the proportion of flowers visited by the two major pollinator groups remained consistent between years in the five localities sampled during 1996 and 1997, as revealed by significant correlations between years for percent abundance of Hymenoptera ($r=0.903$, $N=5$, $P<0.05$) and Lepidoptera ($r=0.902$, $N=5$, $P=0.05$).

Populations differed also in pollinator species diversity, as measured by Shannon's diversity index for the proportional flower visitation data

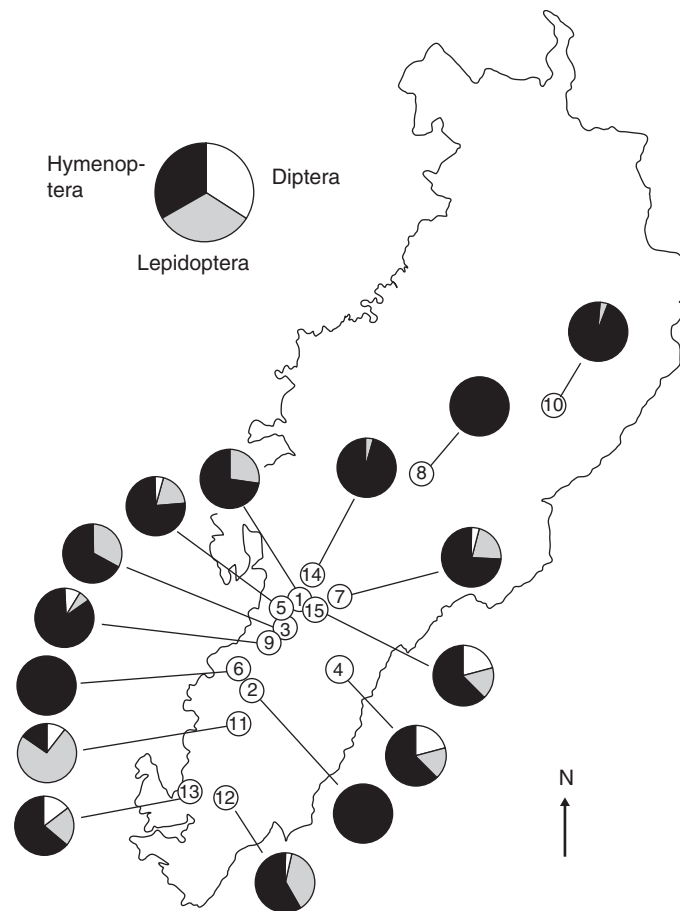


Figure 15.3 Geographical variation in the relative importance of the three main groups of pollinators of *Lavandula latifolia*, estimated by the proportion of total flower visits contributed. Localities are identified by numerals, as in Electronic Appendix 15.2 (<http://www.eeb.utoronto.ca/EEF/>). Locality names, geographical coordinates, and elevations are given in Herrera (2005: Table 2).

for each locality. Diversity correlated negatively and marginally significantly with latitude ($r = -0.498$, $N = 15$, $P < 0.10$). Pollinator abundance, measured as the mean number of flowers visited per 3-min period (all species combined), did not correlate significantly with latitude, for data from either populations ($r = 0.017$, $N = 15$, $P > 0.90$) or individual plants ($r = 0.010$, $N = 300$, $P > 0.80$).

15.4.3. Step 2: Demonstrate pollinator-mediated selection on floral traits

Phenotypic selection on floral morphology via its influence on the maternal component of

pollination was assessed by fitting a generalized linear model to plant means ($N = 300$ plants), with pollen receipt per stigma (mean number of pollen grains; NPG) as the response variable, and the mean lengths of the UL and CT as independent variables. The response variable was \ln -transformed and the analysis considered a negative binomial distribution of errors. Pollen receipt is a good surrogate of maternal fitness, as it correlates strongly with the number of pollen tubes in the style for the flowers sampled ($r = 0.660$, $N = 2987$, $P < 0.0001$; only flowers with $NPG > 0$ included), which in turn affects seed production per flower directly (CM Herrera unpublished data). Among-population variation in phenotypic selection on

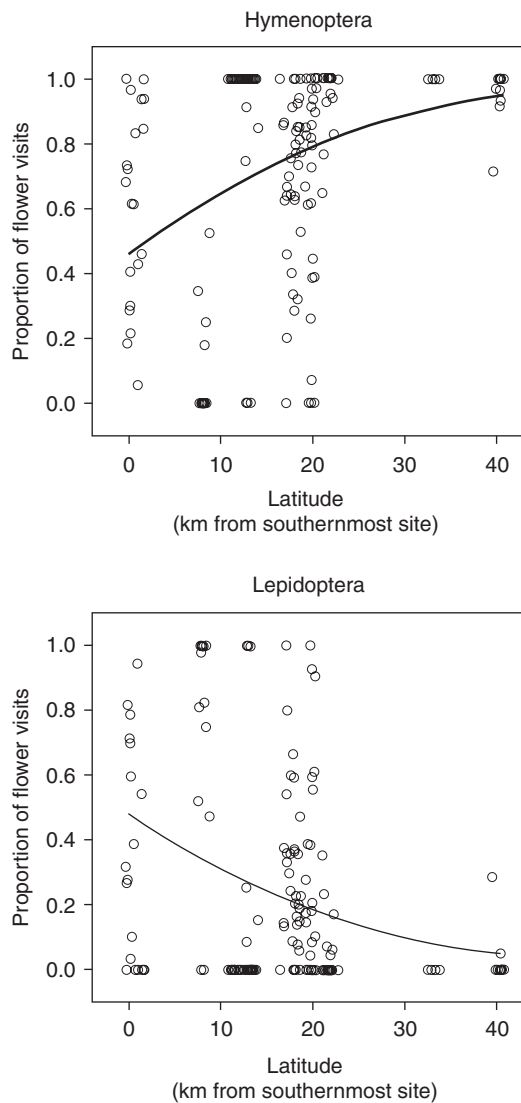


Figure 15.4 Latitudinal variation in the proportion of flower visits contributed by Hymenoptera and Lepidoptera to individual *Lavandula latifolia* plants. Each symbol corresponds to a different plant. Only plants with > 10 flower visits are used ($N = 161$). Logistic regressions are shown as solid lines (generalized $R^2 = 0.12$ and 0.10 for Hymenoptera and Lepidoptera, respectively; $P < 0.0001$ in both cases). A small random deviate was added to latitude data to reduce point overlap.

floral morphology was evaluated by testing the homogeneity of slopes of the relations of pollen receipt to the measures of floral morphology among populations with Population \times UL and Population \times CT interactions (e.g., Strauss *et al.*

2005; Rey *et al.* 2006). For simplicity, we focused only on directional selection gradients and did not assess quadratic terms in the phenotypic selection model, as this approach facilitates interpretation of population \times trait interactions. Restriction of the analyses to directional selection is also justified in the present context, because directional selection seems to play the central role in phenotypic diversification at the species level and above (Rieseberg *et al.* 2002). Nevertheless, the model that we used to test geographical heterogeneity in selection could be extended easily to accommodate tests of heterogeneity in disruptive/stabilizing selection (Strauss *et al.* 2005; Rey *et al.* 2006).

This analysis revealed significant directional phenotypic selection on floral morphology through female function. Pollen receipt varied significantly among plants with the mean length of the UL ($F_{1,255} = 20.52$, $P < 0.0001$), but not with the mean length of CT ($F_{1,255} = 3.61$, $P > 0.05$). The relation between pollen receipt and length of UL differed significantly among populations (Population \times UL interaction, $F_{14,255} = 2.44$, $P < 0.01$), demonstrating population differences in the nature of pollination-mediated phenotypic selection on this trait. Similar variation among populations was not evident for length of CT (Population \times CT interaction, $F_{14,255} = 1.25$, $P > 0.1$). Consequently, we observed significant phenotypic selection only for the length of the UL and this selection varied among populations.

15.4.4 Step 3: Assess geographical divergence in selection

To examine whether the observed variation in selection gradients for the length of the UL has a geographic component, we assessed their correlation with latitude. Generalized linear models were fitted to plant means data separately for each population, and the standardized regression coefficients for UL length obtained from these models (b_{UL} 's hereafter) used as surrogates for phenotypic selection coefficients. b_{UL} increases significantly with latitude ($r_s = 0.671$, $N = 15$, $P < 0.01$; Fig. 15.5), demonstrating a geographical gradient in directional selection on that floral trait over the relatively restricted latitudinal range considered.

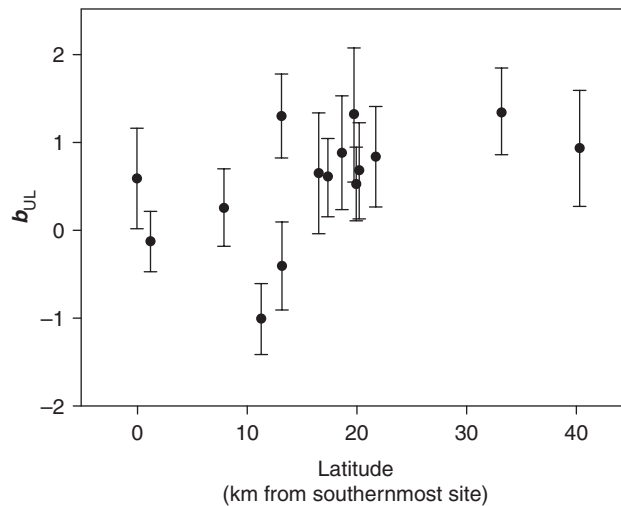


Figure 15.5 Latitudinal cline in pollination-mediated phenotypic selection on the upper corolla lip of *Lavandula latifolia* flowers. Dots represent the phenotypic selection coefficients ($b_{UL} \pm SE$) estimated separately for each locality.

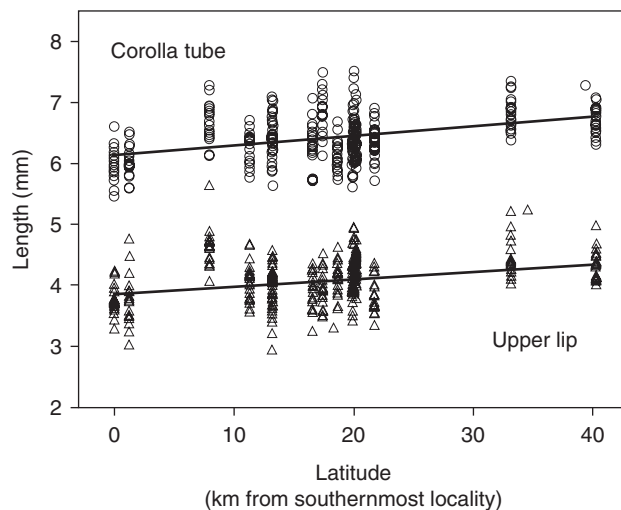


Figure 15.6 Clinal variation in lengths of upper lip (UL) and corolla tube (CT) of *Lavandula latifolia* flowers across the 40-km wide latitudinal range studied. Symbols represent means for individual plants (circles, CT; triangles, UL; $N=300$ plants). Solid lines are least-squares linear regressions (CT: $F_{1,298} = 57.18$, $R^2 = 0.16$, $P < 0.0001$; UL: $F_{1,298} = 32.04$, $R^2 = 0.10$, $P < 0.0001$).

15.4.5 Step 4: Evaluate the match between divergent selection and phenotypic divergence

Corolla size varied gradually with latitude among the *L. latifolia* populations studied. Population means ranged between 3.7–4.6 mm and 5.9–6.8 mm for UL length and CT length, respectively (Electronic Appendix 15.2), with significant differences among populations ($F_{14,285} = 15.26$ and 15.03, for UL

and CT, respectively; $P < 0.001$). Plant means for UL and CT increase significantly from southern to northern locations (Fig. 15.6), indicating a latitudinal cline in corolla size over the geographical range studied. The cline is rather steep, as denoted by average ($\pm SE$) gradients of 0.012 ± 0.0021 mm \cdot km $^{-1}$ and 0.016 ± 0.0021 mm \cdot km $^{-1}$ for UL and CT, respectively, as estimated from the slopes

of linear regressions in Fig. 15.6. These gradients represent changes in population means of about 0.30% and 0.35% per km for UL and CT, respectively. The morphological cline and the pollination-related selection cline are therefore closely congruent, with the largest corollas in populations where directional selection favouring large corollas is strongest.

15.4.6 Step 5: Genetic basis of population differences in floral traits

A rigorous demonstration of adaptive, pollinator-driven, regional differentiation of *L. latifolia* flowers finally requires demonstrating that observed population differences in corolla traits have a genetic basis, rather than resulting from plastic responses to some variable environmental factor. This analysis could be accomplished with common-garden experiments or reciprocal transplants (reviewed by Schluter 2000); however, we have not performed such experiments, nor have most previous investigations relating geographic variation in floral traits to differences in pollinator composition and/or selection patterns. Clearly, such studies, which assess an essential component of natural selection, warrant consideration when planning future investigations.

Circumstantial evidence suggests a genetic component to variation among *L. latifolia* populations. Indirect evidence suggests that regional variation in corolla size is not a plastic response to changing abiotic environment. Soil nutrient availability and water stress can induce plastic variation in corolla size (Villarreal and Freeman 1990; Frazee and Marquis 1994; Galen *et al.* 1999); however, soil nutrient properties do not vary latitudinally among the 15 sites considered in our study of *L. latifolia* (C. M. Herrera, unpublished data). Total annual rainfall does vary latitudinally across the study region ($r = -0.559$, $P < 0.001$; mean rainfall data from $N = 40$ weather stations), but the relation is negative and thus contradicts the expected effects of water stress on latitudinal variation in corolla size. In the absence of relevant environmental variation, we expect that the variation in corolla size that we observed for *L. latifolia* has a genetic component, as has been observed for

other plant species (Worley and Barrett 2000; Galen and Cuba 2001; Lendvai and Levin 2003).

15.4.7 Interpretation and caveats

Adaptive clines are maintained by the opposing interplay between the diversifying effect of variable selection along an environmental gradient and the “homogenizing” effect of gene flow (Slatkin 1985). Although we have demonstrated spatially variable selection, which is consistent with observed phenotypic variation, the observed clinal divergence in floral traits could partly reflect neutral phenotypic differentiation among populations under restricted gene flow and isolation by distance (Endler 1977). Data on amplified fragment length polymorphism (AFLP) markers for three of the *L. latifolia* populations studied here (Populations 1, 8, and 13 in Fig. 15.3) militate strongly against this possibility. These data reveal significant, but quantitatively modest genetic differentiation along the latitudinal range examined ($G_{ST} = 0.062$; 95% credible interval = 0.052–0.072), which is considerably smaller than the phenotypic differentiation for the floral traits that we examined (0.35–0.50; estimated using Spitze’s [1993] formula for Q_{ST} : CM Herrera and P Bazaga unpublished data). If the AFLP markers used are effectively neutral and observed phenotypic differentiation reflects mainly genetic differences, these preliminary results support our interpretation that variable selection, rather than genetic drift, is the main factor maintaining the cline in corolla size (see Merilä and Crnokrak 2001). That CT length varies latitudinally even in the absence of demonstrable phenotypic selection may reflect a correlated response to selection on UL length resulting from the close integration between the two traits (Herrera 2001; see Chapter 14). The similarity in selection gradients for the two traits (Fig. 15.6) supports this interpretation. Therefore, our findings for *L. latifolia* are interpreted most reasonably as indicating a consistent latitudinal gradient in pollinator-mediated selection on corolla lip length through its effects on pollen import, resulting in adaptive intraspecific differentiation in the form of a latitudinal cline in corolla size. However, confirmation of this conclusion awaits

common-garden or transplant experiments (step 5 above).

We have not investigated the proximate mechanisms whereby geographical variation in pollinators governs geographically variable selection on corolla size. As noted earlier, individual species and major groups of *L. latifolia* pollinators differ in incidence and the amount of pollen they deposit on stigmas, and they probably also respond differently to variations in corolla size. Therefore, population estimates of phenotypic selection on floral traits should reflect, in intricate ways, the differences among individual pollinating species in floral preferences, pollen dispersal, local abundance and flower visitation rates (see Eckhart 1991, 1992). With a taxonomically diverse pollinator assemblage, such as that of *L. latifolia*, dissecting the proximate mechanisms involved and the contribution of individual pollinator taxa to observed variation in selection may prove intractable. More positively, our results for *L. latifolia* are among the few to date providing empirical support for diffuse phenotypic selection on a plant trait exerted collectively by a multi-species animal assemblage (Strauss *et al.* 2005). This is an important result, as it suggests that adaptive floral divergence may not require specialization on particular pollinators as traditionally implied (e.g., Stebbins 1970). As shown here, taxonomically diverse pollinator assemblages, despite heterogeneity in pollinating characteristics, may collectively exert net selection on floral traits that, if spatially variable, may promote floral divergence.

15.5 Concluding remarks: towards an improved research programme in floral diversification

Inquiries into intraspecific diversification in floral traits mediated by divergent selection from pollinators represent a subclass of investigations on local adaptation, i.e., adaptive microevolutionary change. Nevertheless, in contrast with the voluminous literature on local adaptation in physiological, morphological or life history traits of plants (Linhart and Grant 1996; Jonas and Geber 1999, and references therein), our literature surveys found few substantiated studies of local adaptation

in floral traits. One reason for this scarcity seems to be that pollination biologists have not always considered geographic variation to be important, as illustrated by scarcity of geographically informed investigations in our literature survey. Knowledge of the interaction of most plants with pollinators is based on single local snapshots of a process that varies among populations. Another reason for the rarity of geographic studies of selection on floral traits is that making a compelling case for pollinator-driven adaptive floral diversification is not easy, as illustrated by our *L. latifolia* study.

Little is known of patterns and processes related to intraspecific floral diversification (also see Chapter 16), so we largely focussed on how it should be studied, rather than on how it operates. Our literature review demonstrates that plant-pollinator interaction in a geographical context is a relatively neglected element of research on floral diversification. Furthermore, the few studies that address intraspecific variation explicitly generally provide ambiguous evidence for a *causal* role of variable selection by pollinators in generating intraspecific differentiation in floral traits, as most rely on correlative evidence alone, which provides the weakest support for adaptive interpretations. Research on floral diversification would benefit from both increased awareness of the central significance of incorporating the geographical context in studies of plant-pollinator interactions and, perhaps more importantly, reduced use of character-pollinator correlations to judge the occurrence of pollinator-mediated intraspecific diversification.

The five-step protocol, exemplified above for *L. latifolia*, may help circumvent some of the most obvious limitations of the few earlier studies on intraspecific floral variation. Particularly, we consider the demonstration of selection (step 2) and its geographical variation (step 3) essential to any investigation of the *current* adaptive value of intraspecific floral diversification. However, three aspects should be considered in relation to the phenotypic selection analyses involved in these steps. Firstly, although we considered only selection through the female function, steps 2 and 3 should also ideally assess possible selection

through the male function, which may or may not mirror selection through female function (Conner *et al.* 1996; Maad 2000; Chapter 14). Secondly, the regression analysis promoted by Lande and Arnold (1983) and implemented in steps 2 and 3 above is not the only way to determine whether pollinators mediate selection on floral traits and document geographical variation. Other approaches to selection analysis, such as those based on path analysis and structural equations modelling, would be equally useful (e.g., Gómez 2000; Rey *et al.* 2006). Thirdly, contemporary measures of selection on floral traits provided by phenotypic selection analyses may provide limited insight into the adaptive origin of floral diversification in species where diversification occurred during past ecological scenarios, promoted by selective regimes different from those operating currently (see Herrera 1996 for some examples and a general discussion on “history-laden” versus “nonhistorical” approaches to the study of floral adaptations). In the Mediterranean Basin, where species originating prior to the appearance of Mediterranean climate conditions coexist with recent lineages evolved under current ecological conditions (e.g., Herrera 1992, Verdú *et al.* 2003), this limitation probably applies more importantly to phenotypic selection analyses of species that evolved before the appearance of Mediterranean climate conditions (e.g., *Viola cazorlensis*; Herrera 1990, 1993) than to those of species evolved under current ecological conditions (e.g., *Lavandula*).

The protocol that we propose focuses on the stepwise testing of an explicit *a priori* prediction: if variable pollinators are a major influence on floral diversification, then geographic variation in the abundance of pollinators with different floral preferences and pollinating quality (step 1) should impose geographic variation in selection on floral traits (steps 2 and 3), eventually causing phenotypic floral divergence (step 4) with a genetic basis (step 5). The sequence of steps of the proposed protocol reverses the inferential *a posteriori* approach typically applied to test links between intraspecific floral diversification and pollinator variation. The traditional approach can proceed beyond correlative evidence only with difficulty, leaving little room for incorporating explicit

cause–effect hypotheses about selection, and it is susceptible to ad hoc hypothesis accommodation and hypothesis fudging (sensu Lipton 2005). In contrast, the approach illustrated here for *L. latifolia* tests the central elements of adaptive interpretations of floral diversification explicitly in a stepwise manner, running from putative causes to purported effects, and is thus less susceptible to accommodation and fudging. There are reasons for predictions counting more than accommodations (Lipton 2005) and also, therefore, for preferring a prediction-based, deductive logic when assessing the role played by pollinators in intraspecific floral diversification.

Acknowledgements

C Alonso, MC Anstett, A Benavente, D Ramírez, R Requerey, L Sánchez, and A Tíscar assisted with the *Lavandula* study in the field and/or the laboratory. P Bazaga shared unpublished genetical data, and two reviewers provided valuable suggestions. MCC was supported by a postdoctoral grant from the Ministerio de Educación y Ciencia, and MM by a I3P Programme postdoctoral contract from the Consejo Superior de Investigaciones Científicas.

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