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Spatial variation in selection in a plant-pollinator system in the wadis of Sinai, Egypt

Abstract We studied an insect-plant pollination system in adjacent steep-sided wadis and a connecting plain in the mountains of southern Sinai (Egypt): this environment creates a strongly divided habitat, which may promote the local differentiation of sub-populations. We tested for spatial differences in phenotypic reproductive characters of the only plant flowering abundantly in early spring, *Alkanna orientalis* (Boraginaceae), and its major pollinator at that time of year, *Anthophora pauperata* (Apoidea, Anthophoridae). There were significant morphological differences between sub-populations of *Alkanna*, mainly between plants from the narrower wadis and those on the interconnecting plain. Flowers on the plain were larger, with wider corollas and more nectar standing crop; these plants retained more flowers on the inflorescence, but received many fewer visits to flowers. There was a significant selection gradient between flower size and maternal fitness (seed set) in the plain, but not elsewhere. Natural selection may have increased resources devoted to attracting insect visitors in response to fewer pollinating visits in the plain. Consistent with this explanation, by experimentally manipulating flower number per plant, we showed that within a wadi having more flowers on a plant secured more visits.

Key words Phenotypic selection · Floral morphology · Pollination · Solitary bees · Foraging

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Introduction

The selection exerted by pollinators on plants is in some ways a particularly accessible system for assessing the adaptive significance of reproductive traits. We can distinguish between selection and the evolutionary response to selection, and argue that the existence of selection among phenotypes allows us to investigate the evolutionary significance of phenotypic traits (Lande and Arnold 1983; for a justification, see Herrera 1993). This approach has been widely used in documenting the evolutionary significance of within-population variation in different traits related to plant reproduction (e.g. Herrera 1993; Stanton et al. 1991), and using plants for this has a number of advantages (Primack and Kang 1989).

In pollination studies, there is now a new emphasis on experimentation (e.g. Bell 1985), estimating adaptive landscapes (Armbruster 1985, 1990), and on considering individual variation (Herrera 1990a, 1991, 1993; Murcia 1990; Young and Stanton 1990; Stanton et al. 1991; Eckhart 1991). With new multivariate techniques for measuring selection gradients (Lande and Arnold 1983), selection on different floral components can be estimated (e.g. Johnston 1991), and spatial (Stewart and Schoen 1987) and temporal (Schemske and Horvitz 1989) variation in selection quantified.

We are interested in looking at the spatial continuity of selection, at the direction and strength of selection in adjacent sub-populations of a plant-pollinator relationship; Stewart and Schoen (1987) have shown already that the direction of phenotypic selection can be inconsistent at different localities, even those separated by only a few metres. We might predict at least the potential for such differences in places where adjacent populations are strongly subdivided by topography. In the mountains of southern Sinai (Egypt), plants grow in steep-sided rocky wadis (valleys) separated by relatively high ridges; wadis feed into other wider wadis, eventually reaching the coastal plain. Plant sub-populations growing in wadis are often linked via connecting sub-populations at the mouths of the wadis.

We address here the question of whether sub-populations in adjacent wadis are phenotypically different in characters of relevance to pollination, and if so, whether the differences are likely to be caused by differences in the plant-pollinator relationship.

Materials and methods

Study area

Fieldwork was carried out during March and April 1992 at the Field Station of Suez Canal University in St. Catherine, Sinai (see Dewedar 1991; Willmer et al. 1994). St. Catherine lies surrounded by mountains in a broad open wadi (the Plain of El Raha, henceforth called "the Plain") at 1600 m above sea level: from the Plain there radiate three narrow, steep-sided wadis named Tofaha, Arbaein and Dir (Fig. 1). Nearly all our fieldwork was done on the Plain and in Dir, Arbaein and Tofaha: in this report all four areas are called "wadis". We used three (approx. 100 × 100 m) sampling sites in the wadi bed of Arbaein, at 50 m (the wadi "mouth"), 1200 m ("middle") and 2500 m ("upper") from the entrance. In contrast, we sampled continuously along the wadi beds of Dir and Tofaha for distances of 1700 m and 800 m respectively from their entrances; in some cases in the results we have divided these continua arbitrarily into base, middle and upper sites in these wadis.

Fig. 1 Map of the St. Catherine study site in the mountains of southern Sinai, Egypt. The study sites are marked: *1* Plain, *2* Wadi Dir; *3* Wadi Arbaein, *4* Wadi Tofaha
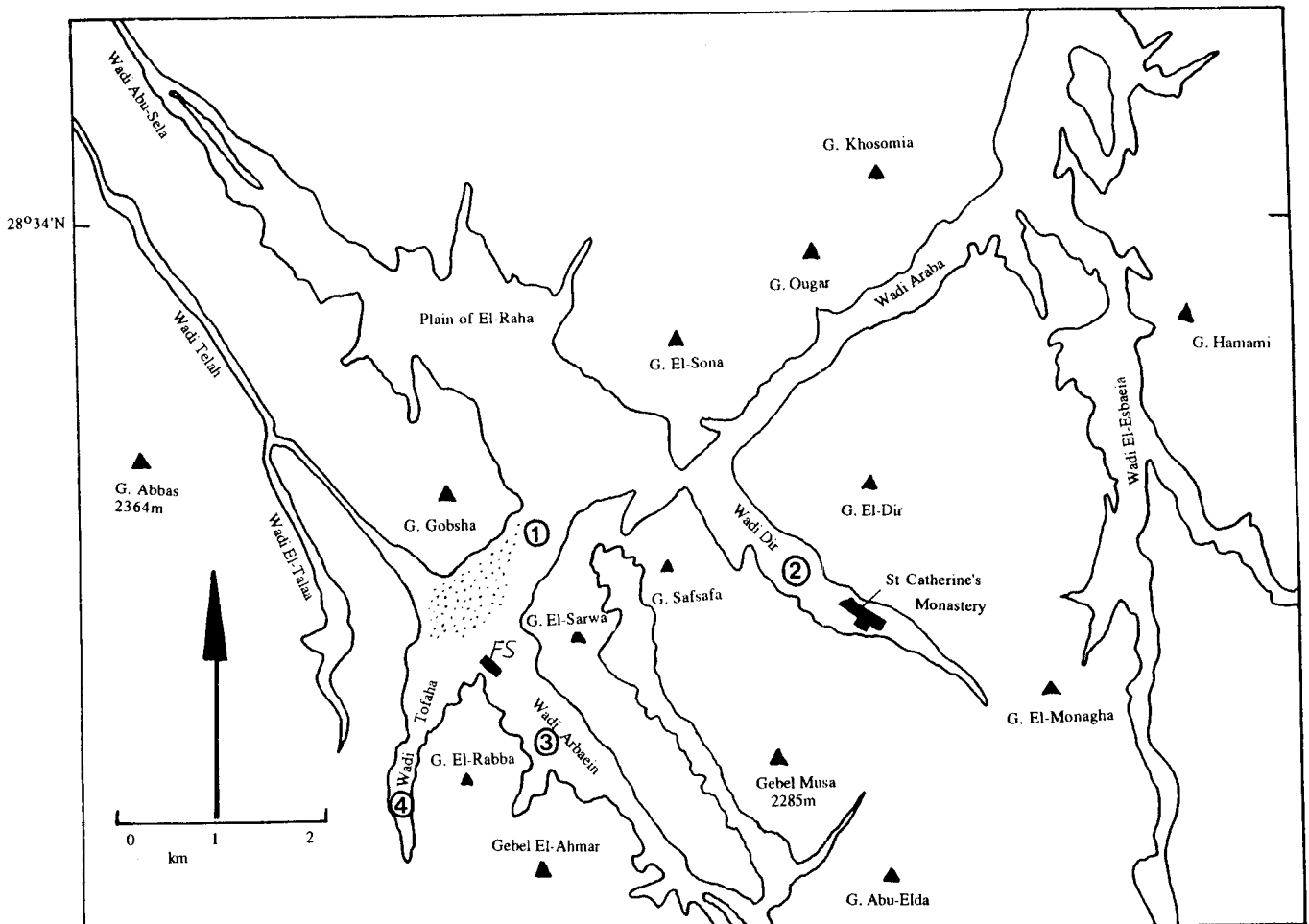
Tofaha is the shortest, narrowest and steepest of the wadis, and subjectively the most isolated from all other wadis, whilst Dir starts broad and flat and is subjectively less isolated from the Plain than are the other two wadis; Arbaein is intermediate.

The system studied

In March/April the flowering season had only just begun, and only a single plant species was flowering in profusion: this was *Alkanna orientalis* (L.) Boiss. (Boraginaceae), a plant confined within Egypt to southern Sinai mountains (Täckholm 1974; Mansour and Saleh 1986). *Alkanna* has most species in the Aegean Islands (Greece) and Anatolia (Turkey) (Rechinger 1965, Davis 1978). There is some taxonomic confusion over *A. orientalis*, which occurs from Greece to Egypt, with an outpost in Algeria (Polunin and Huxley 1972); the Greek populations may belong to separate species (Rechinger 1965) or not (Tutin et al. 1972), and the taxonomic status of the Sinai populations seems never to have been sorted out definitively.

A. orientalis is a fairly common plant in the Sinai mountains, but grows only at the highest elevations above about 1500 m. It is a short-lived perennial that flowers in its second year of growth (Semida 1994), and is covered with sticky irritant glandular hairs very effective in preventing the leaves from being eaten by goats and camels, which only eat it when dry. The relatively large, yellow, trumpet-shaped, protandrous flowers are borne on a determinate scorpioid cincinnus, and have bilocular anthers at two levels within the flower (three upper and two lower anthers) and a single gynobasic style. The plant appears to reproduce only by seed, and is an obligate outcrosser (see below).

Plants in the three sites in Arbaein were distributed more or less randomly up to 30 m above the wadi bed, becoming much less com-

mon above this but continuing right up to the mountaintops. At the mouth, middle and upper parts of Arbaein, we calculated densities of 235, 473 and 653 plants ha^{-1} respectively: we did not measure densities quantitatively in the Plain, but they were lower than the mouth of Arbaein. The number of flowers open on an individual plant varied from zero to more than 500. From the growing tip of the inflorescence down the spike, there was at any one time one bud, a mature flower, and 0–5 old flowers. Flower buds opened at all times of the day. Each flower lasted for only 1–2 days (usually 2) as a mature yellow flower, before either withering or turning white. Only mature buds and mature flowers were visited by insects.

Virtually the only visitor to the flowers through most of the study period was a solitary bee, *Anthophora pauperata* Walker (Apoidea, Anthophoridae) (bees determined by Dr. D.B. Baker, Hope Museum, Oxford) (see also Willmer et al. 1994, where the bee is called *Anthophora* sp.nov.). The flowers were visited occasionally by the much larger *Anthophora caelebs* Grib. Towards the end of the study period a few hoverflies [*Eupeodes* (*Metasyrphus*) and *Scaeva* spp.] and bombyliids (*Orthoprosopa* sp., *Exoprosopa* sp.) became occasional visitors. *Alkanna* has a long main flowering period, from April to June, but *Anthophora pauperata* appear to switch to other flowers as soon as they appear, half-way through the flowering season of *Alkanna*: bombyliids and occasionally Lepidoptera then become the main but infrequent visitors to its flowers (Semida 1994).

Morphological data

We obtained two datasets on floral morphology: the first was corolla depth and width from at least three *Alkanna* flowers per plant from at least 100 plants per wadi, measured in the field using vernier calipers. Sampling 15 plants from each site and using a binocular microscope, we obtained further data for five branches (three flowers on each) from each plant, measuring corolla width, corolla depth, the height of both sets of anthers, the length of the style, and seed set down the longest inflorescence.

Nectar availability and seed production

We measured the nectar availability (standing crop) from flowers in the field using 1-μl Drummond microcapillary tubes. We gently removed nectar from the back of picked flowers and measured the volume and concentration [in gm sucrose equivalents per 100 gm solution using Bellingham and Stanley (Tunbridge Wells, Kent, UK) refractometers adjusted for small volumes]. Flowers that we selected for nectar measurement had not been bagged, but were continuously open to insect visitors before being picked. Where appropriate, we converted these data to the weight of nectar sugar (in μg sucrose equivalents: Weast 1985). Relative to many other plant species, *Alkanna* flowers secrete a substantial amount of nectar, with up to 6 μl at a fairly constant concentration of about 35% sucrose-equivalents.

Seed production could be scored from about the fourth calyx below the mature flower: there are up to four developing ovules. We sampled the longest flowering spike from plants, and scored seed production in each flower down to what we assume to have been the first flower of that plant. Few flowers set the maximum of four seeds, and many failed to set any.

Flower phenology and anthesis

Forty flowers, five per plant on four plants in Wadi Arbaein and four plants on the Plain, were marked as buds just beginning to open. They were censused every day until all had fallen. Anthesis was scored separately in each locule of each anther for closed buds and flowers in each of the above stages. Anthesis occurs almost immediately the bud begins to open, and pollen is fully available in flowers that have only just started to open: pollen-collecting female *Anthophora* concentrate upon this flower stage (see Willmer et al. 1994).

Visits to flowers

In order to test for the effects of flower number on the frequency of visits by *Anthophora*, we manipulated the number of flowers per plant experimentally in each wadi, generating at least five replicate plants bearing 12, 25, 50, or 100 flowers; then simultaneously in all wadis we observed the number of visits during 10-min observation periods over most of 1 day. We counted separately the inspections and flower visits to plants by male and female *Anthophora pauperata* and other visitors: nearly all visits were by *A. pauperata*.

We tested whether *Alkanna* was outcrossed by using 80 bagged flowers randomly allocated to the treatments of hand cross- or self-pollination. We tested the *a priori* prediction that cross-pollinated flowers set more seed using the specific version of a non-parametric ANOVA (see Meddis 1984; Barnard et al. 1993), tested with a Z-test. In addition, we bagged selected flowers from opening, and then allowed a single visit from a female *Anthophora* or from a bombyliid fly *(Exoprosopa* sp.*)*; the singly visited flower was then tied up to prevent more visits, and we measured the seed set by this flower (*E*) and the next one to flower (*A*) (which was left open to all insect visits during its lifetime). The *a priori* predictions that $A > E$ in each case (for bees and for flies), and that bee seed set > fly seed set, were also tested using specific non-parametric anovas. Finally, we could perform one type of test for short-term pollen limitation by testing the *a priori* prediction that hand cross-pollinated flowers should set more seed than naturally pollinated flowers.

A small experiment tested whether flowers responded to being visited by altering the rate of nectar secretion. 300 flowers of one large plant were allocated treatments of: (A) controls (simulating unvisited flowers); (B) upper anthers removed (simulating visitation and crudely altering pollen reward); (C) using a fine paintbrush to simulate bee feeding action (probably removing both pollen and nectar); and (D) removal of nectar with a fine microcapillary.

Statistical analysis

We used Statgraphics Plus v.7 (Manugistics Inc., Rockville, Maryland, USA) for most of the analyses. We checked for homogeneity of variances in all anovas: where these were not homogeneous, we used the non-parametric equivalent (Kruskal-Wallis test, denoted by the KW test statistic). All mean values are cited as ± 1 SE: "ns" means $P > 0.05$.

When several morphological variables are measured per individual, they are usually intercorrelated because there are large and small individuals. Therefore we usually replaced the original variables with the uncorrelated size and shape variables derived from principal components analyses (PCA). The first axis of a PCA usually explains most of the variation, and is a size factor in cases where all the coefficients have the same sign and approximately the same magnitude: subsequent axes are normally shape factors. Scores along the PCA axes become the new uncorrelated set of variables. For example, in order to separate size from shape features we ran a PCA on field-measured data on corolla widths and depths: this did indeed separate size from shape, since the first axis, PCA1, has 73% of the variation and same-sign coefficients of equal magnitude (= size), whereas PCA2 has 27% of the variation and opposite-sign coefficients (= a shape factor).

When multivariate data are separated *a priori* into groups (e.g. the different wadis), an appropriate technique for discovering whether there are significant differences between those groups is discriminant function analysis (DFA). This finds axes of independent variation that maximise the differences among the groups (more precisely, the ratio of the within-group to the among-group variance).

To test for spatial differences in phenotypic selection, we used analysis of covariance using GLIM (NAG Software, Oxford, UK). Selection gradients would usually be calculated as the standardized partial regression coefficients of size and shape variables on

maternal fitness (cf. Lande and Arnold 1983), but here we used the results from the ANCOVA because only a single size or shape variable entered the stepwise regressions we ran.

Results

Were there spatial differences in floral morphology ?

Were there differences among wadis ?

There were large differences among the wadis in the mean values of many of the morphological variables (Table 1). The multivariate test for wadi differences, the first axis of the DFA, was very highly significant ($\chi^2_{15} = 77.6$, $P \ll 0.001$, containing 94% of the within/among group variation), and contrasted flowers from the Plain (wide corollas and short styles) with flowers in other wadis (narrow corollas and long styles). Arbaein and Tofaha had relatively small, wide and short flowers; Dir and the Plain had relatively large flowers. Thus there were indeed strong morphological differences, mainly between the Plain and the other wadis.

There were other differences too (Table 1). In Wadi Arbaein only 25% of flowers turned white before withering and dropping, and all flowers had withered and dropped by the morning of the 4th day after opening: in contrast, on the Plain, 85% of flowers turned white and lasted for at least 1 extra day before dropping. This created a very different appearance in the different wadis, because there were many more flowers on an inflorescence at any one time in the Plain population (KW = 71.1, $P \ll 0.001$), and there were also more pale flowers per
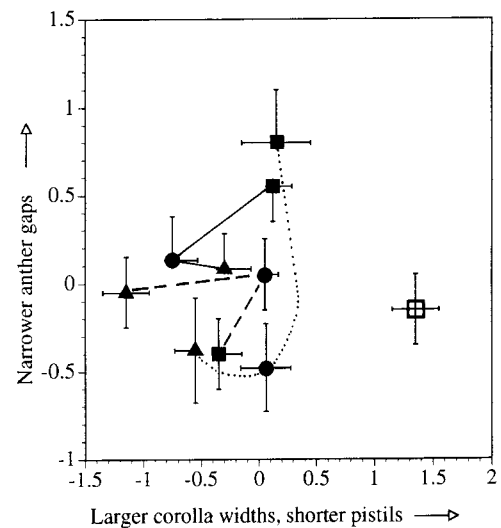

Larger corolla widths, shorter pistils ⟶

Fig. 2 Plot of the scores along the first two axes of a discriminant function analysis of ten groups (three sites within each of three wadis, plus plants from the Plain). *Lines* connect sites within wadis: *dots* Dir, *dashed* Arbaein, *solid* Tofaha. *Symbols* indicate sites: *squares* wadi mouth, *circles* middle, *triangles* upper wadi. The *open square* is data for the Plain

plant on the Plain. An analysis of covariance looked for differences between wadis in the number of pale flowers per plant, allowing for the total number of flowers. Plants on the Plain and in Dir had more pale flowers, and those from Arbaein and Tofaha fewer pale flowers, for a given total number of flowers ($F_{3,180} = 17.6$, $P \ll 0.001$).

In addition there were more open flowers per plant for a given size of plant in the plants growing on the Plain

Table 1 Morphological and reproductive characteristics of *Alkanna orientalis* in different locations. Anther positions were measured from the flower base, where it is connected to the inflorescence. Relative to the height of the lower anthers, the upper anthers are on average at 1.32 times this distance, and the corolla mouth at 2.0. Style heights are variable, with a multimodal distribution peaking at relative heights of 1.0, 1.2 and 1.4, and a few flowers with styles at 1.6 times the height of the lower anthers. This variation is not age-dependent: thus *Alkanna* may be tristyl-

ous. All morphological measurements are in mm; for the percentage seed set, flower numbers and nectar measurements, sample sizes are smaller (between 40 and 100) and the means are given with their the standard errors. *N* sample size, *CD* corolla depth, *CW* corolla width, *UA* height of upper anthers, *LA* height of lower anthers, *SL* style length,*%S* percentage seed set, *CV* coefficient of variation, *Flowers* number of flowers per inflorescence, *Pale flowers* number of pale flowers per inflorescence, *Nectar* sugar per flower µg

	N	CD	CW	UA	LA	SL	%S	Flowers	Pale flowers	Nectar
Overall	740	11.6	8.4	7.7	5.8	6.8	20.9±1.0	2.5±0.1	4.3±0.4	–
Minimum		*7.2*	*4.6*	*4.0*	*3.0*	*3.7*	*0.0*			
Maximum		*15.2*	*12.7*	*9.9*	*7.8*	*10.8*	*63.6*			
CV		*8.9*	*14.7*	*9.6*	*11.1*	*15.7*	*66.7*			
Plain	115	12.3	9.4	8.2	6.2	6.9	12.6±1.8	3.2±0.1	7.7±0.6	8.4±0.9
Arbaein	273	11.6	8.3	7.6	5.7	6.9	23.0±1.7	1.8±0.1	0.6±0.3	–
Upper	*52*	*11.2*	*7.7*	*7.4*	*5.6*	*6.8*	*20.4*			
Middle	*105*	*11.9*	*8.7*	*7.7*	*5.9*	*7.1*	*18.5*			
Mouth	*116*	*11.5*	*8.1*	*7.6*	*5.7*	*6.6*	*29.5*			
Dir	158	11.7	8.4	7.7	5.8	6.8	17.5±1.8	2.1±0.1	3.8±0.4	3.8±0.5
Upper	*42*	*11.8*	*7.9*	*7.8*	*5.9*	*6.5*	*20.8*			
Middle	*58*	*11.9*	*8.6*	*7.9*	*5.8*	*7.2*	*18.5*			
Mouth	*58*	*11.2*	*8.6*	*7.4*	*5.8*	*6.6*	*13.3*			
Tofaha	177	11.3	8.1	7.4	5.7	6.7	28.3±2.0	2.5±0.2	1.0±0.4	–
Upper	*61*	*11.3*	*8.0*	*7.4*	*5.7*	*6.3*	*38.2*			
Middle	*58*	*11.1*	*7.7*	*7.4*	*5.7*	*6.7*	*26.3*			
Mouth	*58*	*11.3*	*8.6*	*7.5*	*5.7*	*7.0*	*20.3*			

and in Tofaha. We fitted regressions of the form $Y = \exp(a + bX)$ (where Y = number of open flowers, X = plant diameter in cm) to data from each wadi; all regressions were significant, and predicted that, for example, for a 50-cm-diameter plant, the number of open flowers was: Arbaein 29, Dir 74, Tofaha 106, Plain 105. Plants from Arbaein and Dir had fewer additional flowers for a given increase in plant size than Tofaha and those from the Plain (by comparing b values in the regressions, which were significantly different by an *a posteriori* LSD test). Plant size had no effect on floral morphology (e.g. in Arbaein, corolla depth vs. plant size, $r = 0.36$, $n = 24$, ns; corolla width, $r = 0.02$, $n = 24$, ns).

There was a substantial difference in nectar standing crop (KW = 15.3, $P \ll 0.001$) between plants from the three steep-sided wadis, and those from the Plain (see Table 1).

Since each flower can set a maximum of four seeds, for the 45 plants from each wadi we calculated the seed set (%). There were substantial differences between sites in seed set (KW = 25.8, $P \ll 0.001$), with plants on the Plain setting the least, Tofaha the most, and Arbaein and Dir intermediate (Table 1).

Were there differences among sites within wadis ?

A further DFA with ten groups consisting of sites within wadis (base, middle, top of each wadi, plus the Plain population) looked for consistent patterns of site differences within wadis. There were two significant discriminant functions; the first ($\chi^2_{45} = 150.6$, $P \ll 0.001$) was essentially the same as before, contrasting flowers having wide corollas and short styles with flowers having narrow corollas and long styles; this contained 67% of the within/among-group variation. The second axis ($\chi^2_{32} = 55.8$, $P < 0.01$) contrasted flowers having narrow gaps between anthers with those having large gaps, and contained a further 17% of the variation. As before, the first discriminant function highlighted the difference between the Plain and all others, whereas the second mainly contrasted the sites at the mouths of wadis Dir and Tofaha against the rest (Fig. 2); flowers from Arbaein populations showed no consistent differences. We conclude that there were some small but inconsistent differences among sites within wadis, over and above the differences among wadis.

Using field-collected data on corolla widths and depths, linear regression showed that there were some overall patterns along the length of the narrow wadis (Dir, Arbaein and Tofaha); these are weak, in the sense that they account for very little of the variation. Flowers became larger down wadis [size (PCA1) vs. distance along wadis, all wadis lumped together, $F_{1,334} = 32.9$, $P \ll 0.001$, $r^2 = 0.09$], and also the corollas became relatively wider and shorter (shape vs. distance, $F_{1,334} = 9.0$, $P < 0.01$). In separate analyses for each wadi, we found that these trends were significant for Dir and Tofaha, but not for Arbaein (for size, $F_{1,52} = 0.44$, ns: for shape, $F_{1,52} = 0.13$, ns), although

flowers from plants at the mouth of Arbaein did tend to be larger with wide corollas relative to plants from the middle or upper wadi.

Spatial variation in phenotypic selection

Using PCA on mean values per plant as morphological variables, we derived new size and shape variables [see Table 2: the first axis (PCA1) was largely a size axis; the second (PCA2) measured increases in corolla width and style length relative to the other variables]. To test for spatial differences in selection, we ran an ANCOVA (see Table 3) using the percentage seed set (standardized within wadis) as the (maternal) fitness dependent variable, flower size (PCA1) and the main flower shape component (PCA2) as the morphological covariates, and 'wadi' as a factor. This demonstrated that there were indeed differences among wadis in the slope of the selection gradient (Table 3). Among individual plants growing on the Plain there was significant phenotypic selection for large flower size, seen in no other site (Fig. 3 and Table 3). In Tofaha there was a significant selection gradient on flower shape: flowers with narrow corollas and short styles set more seed (Table 3). If all plants belonged to a single panmictic population, then there might be phenotypic selection for smaller flower size (see Fig. 3), but this merely reflects the fact that plants from the Plain had the largest flowers, yet set the fewest seeds.

What might cause these differences in floral morphology ?

One hypothesis to explain the differences in plant morphology is that pollinator-mediated selection differs in the different wadis. If true, this predicts that *Alkanna* is an obligate outcrossing species; and (if selection is via maternal reproductive success) that it may suffer from pollen limitation (although pollinator-mediated selection may still occur through variation in male reproductive success, despite no pollen limitation). The bagging experiment in Arbaein tested whether hand cross-pollinated flowers set more seed than hand self-pollinated flowers. The results showed that hand cross-pollinated flowers set an average of 0.8 ± 0.1 seeds, whereas hand self-pollinated flowers set virtually no seeds (0.15 ± 0.1: test for difference $Z = 2.15$, $P < 0.05$). Seed weight was not affected ($Z = 1.33$, $P > 0.05$). A single visit to a flower by female *Anthophora* set an average of 0.46 ± 0.16 seeds ($n = 26$), significantly lower than the number of seeds set by the next flower to bloom (0.83 ± 0.20) that was open to all visitors ($Z = 2.06$, $P < 0.05$). A single visit from the bombyliid fly *Exoprosopa* sp. set 0.14 ± 0.09 seeds ($n = 14$), significantly lower than the 0.7 ± 0.25 set by the flower next to bloom ($Z = 2.1$, $P < 0.05$). Although as predicted, seed set was higher for *Anthophora* than for the bombyliid fly, the difference was not significant ($Z = 1.05$, $P > 0.05$).

Table 2 The results of a principal components (PC) analysis of five morphological variables measured on flowers of *Alkanna orientalis*. The data used were the mean values of each plant, rather than the individual measurements for each flower, and from all the four wadis. The first axis is mainly a size axis since the coefficients are all positive and approximately equal; subsequent axes are shape components of the morphological variation

	Principal component axes				
PC axis	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5
% Variation	60.3	19.1	13.8	5.0	1.8
Variable	Coefficients				
Corolla depth	+0.52	−0.10	−0.04	−0.80	+0.29
Corolla width	+0.31	+0.60	−0.72	+0.16	−0.03
Upper anthers	+0.54	−0.28	+0.04	+0.10	−0.79
Lower anthers	+0.51	−0.35	+0.03	+0.57	+0.54
Style length	+0.29	+0.65	+0.70	+0.08	+0.02

Table 3 Testing for spatial differences between four wadis in phenotypic selection in *Alkanna orientalis*. Using an analysis of covariance on maternal fitness (percentage seed set standardized within each wadi), we fitted *wadi* as a factor with 4 levels, and flower *size* (PCA1) and the main *shape* component (PCA2, corolla width and style length varying independently of corolla depth and anther heights – see Table 2) as covariates. We fitted the following components to the model: *wadi*, the interaction *wadi* × *size* (i.e. fitting separate regressions in each wadi, and looking for differences in the selection gradient between wadis), and *wadi* × *shape* (similarly separate regressions). *Size* and *shape* were not fitted overall since the fitness variable was standardized within wadis (and hence by definition has a mean of zero in each wadi); their interaction was also not fitted since by definition these PCA components are uncorrelated. Significance was assessed by an *F*-ratio (see Crawley 1993)

Source	df	F	P
Wadi	3	0.51	ns
Wadi × size	3	5.67	<0.01
Wadi × shape	3	4.88	<0.01
Error	160		

Parameter	estimate	SE	t	p
Estimates of the final model:				
Constant	−0.05	0.08	0.56	ns
Selection coefficients on size:				
Arbaein	−0.02	0.09	0.21	ns
Dir	+0.05	0.08	0.69	ns
Tofaha	−0.03	0.09	0.35	ns
Plain	+0.32	0.12	2.56	<0.01
Selection coefficients on shape:				
Arbaein	+0.10	0.15	0.68	ns
Dir	−0.09	0.16	0.55	ns
Tofaha	−0.32	0.15	2.16	<0.05
Plain	−0.14	0.23	0.61	ns

Short-term pollen limitation, as tested by comparing hand cross-pollinated flowers with naturally pollinated flowers, failed to reveal any significant difference ($Z = 0.2, P > 0.05$).

Spatial variation in selection might arise if there are differences in the frequencies of visits to flowers, adjusted for the differences in flower number per plant. There was indeed a (curvilinear, exponential) positive effect of flower number per plant on the number of visits by *Anthophora* bees, significant in every wadi (Dir, $r = 0.40$, $P = 0.05$; Arbaein, $r = 0.55$, $P < 0.01$; Tofaha, $r = 0.83$, $P < 0.001$) except the Plain ($r = 0.1$, $n = 24$, ns). Thus
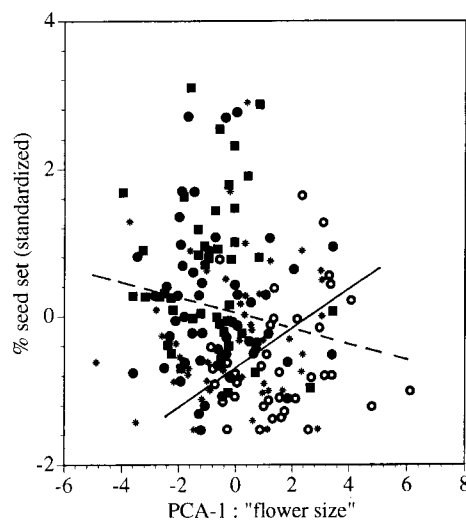

Fig. 3 The standardized percentage seed set versus flower size (score along PCA1) for the four different sites, coded as Plain (*open circles*), Dir (*stars*), Arbaein (*filled circles*) and Tofaha (*squares*). Regression lines are only fitted to significant relationships, i.e. all the points together (*dashed line*), and just to the data for the Plain (*solid line*). These demonstrate the negative relationship overall, but the positive relationship between flower size and seed set for the Plain

having more flowers on a plant attracts more visits from bees. Overall, the mean rate of visiting flowers differed substantially between wadis (KW = 30.8, $P \ll 0.001$), as did the total number of inspections and visits (KW = 30.4, $P \ll 0.001$). There was a very low rate of visits to flowers in the Plain, whereas visitation rates were much higher in Arbaein and Tofaha (Table 4): Dir was intermediate. This matches the morphological differences between flowers of different wadis, e.g. corolla widths, or the score along DFA1 (Table 4). The proportion of seeds set was positively correlated to the mean number of visits ($r = 0.97$, $n = 4$, $P = 0.014$ one-tailed) and negatively to the mean score on DFA1 ($r = −0.90$, $n = 4$, $P = 0.05$ one-tailed). With only four sites it is difficult to draw conclusions, but the pattern is consistent with pollinator-mediated selection leading to the observed morphological differences; the critical prediction is the within-wadi selection gradient (see above).

In Wadi Arbaein we studied the relationship between nectar standing crop and flower morphology among

Table 4 Summary of the some of the differences between wadis in the morphology of *Alkanna orientalis*, and the behaviour and morphology of its pollinator, the solitary bee *Anthophora pauperata*. The mean DFA1 score represents the principal difference be-tween wadis in the multivariate space of the five floral morphological variables – the more positive the score, the wider the corolla and the shorter the style length

Wadi	Bee visits per 10 min	Male head width (mm)	Corolla width (mm)	DFA1 score	% seed set
Plain	1.1±0.3	4.51±0.15 (6)	9.4±0.1	13.4±2.0	12.6
Dir	7.0±4.6	4.71±0.03 (21)	8.4±0.1	−0.3±0.6	17.5
Arbaein	19.5±6.0	4.78±0.02 (35)	8.3±0.1	−4.1±0.7	23.0
Tofaha	22.2±7.3	4.81±0.02 (53)	8.1±0.1	−3.7±0.7	28.3

plants. There was a linear relationship between the average size of flowers on a plant (mean PCA1 score) and mean nectar volume ($r = 0.48$, $n = 34$, $P < 0.01$), but no relationship between mean flower shape (PCA2 score) and nectar volume ($r = 0.18$, ns). Thus flower size could present a reasonable morphological cue for foraging bees, indicating the presence of more nectar.

For Wadi Arbaein, we examined briefly two possible simple environmental causes for differences in flower morphology, i.e. position in the wadi (possibly correlated with water availability) and amount of sunlight. Neither average flower size ($F_{1,25} = 0.08$, ns) nor flower shape ($F_{1,25} = 0.17$, ns), nor indeed nectar standing crop per flower ($F_{1,25} = 0.65$, ns) of plants were associated with distance up the wadi sides. In addition, there were no differences between north- and south-facing sides (F-values 1.78, 0.19 and 1.61 respectively; all non-significant), which receive very different amounts of sunlight.

It is possible that the differences in nectar standing crop and flower longevity between flowers in the Plain versus the three steep-sided wadis could be caused partly by the much lower visitation rates in the Plain, where *Anthophora pauperata* is less abundant and is much less or is not territorial (see Willmer et al. 1994). This could explain the higher rewards left in Plain flowers, and also the lower rates of flower abscission if flowers are commonly knocked off by foraging bees. In the experimental test of the effects of visitation on nectar rewards, after 24 h nectar volumes and concentrations were both lower (0.15 ± 0.04 µl; 35.4 ± 0.8% sugar) in the previously sampled group D than in the control flowers of group A (0.33 ± 0.13 µl; 40.4 ± 1.2% sugar). This difference (50% reduction) is of a similar magnitude to the observed difference in standing crop between Wadi Dir and the Plain. *Alkanna* plants were clearly capable of further secretion after being emptied, so that lower nectar rewards in the flowers of the wadis may not have been "simply" due to extra bee visitors removing the nectar, although this remains the most parsimonious explanation. There was no effect of visitation on flower persistence, however, since 48, 55, 38 and 40% of flowers were lost in each treatment respectively ($\chi^2_3 = 3.0$, ns). Flower visitation rates therefore may be the explanation for observed differences in nectar reward among wadis, but cannot explain equivalent differences in flower retention time. Visitation rates clearly cannot, in any case, be an explanation for the differences in flower morphology.

A final possibility is that floral morphology changes with plant age or phenology, and that we were actually recording the effects of overall age or phenological differences among wadis. From preliminary data (P. Will-mer, unpublished work), there do appear to be age and seasonal changes in flower size, but these account for a very small part of the variation in flower size. They are unlikely to account of the observed overall morphological differences among wadis.

Were there differences in bee morphology between wadis ?

We sampled *Anthophora pauperata* bees from all the sites [sample sizes between 8 (Plain) and 79 (Tofaha)] and measured two morphological parameters, head width and wing length. There were significant differences among sites in the males (e.g. head width, KW = 21.5, $P < 0.001$) but not in females (e.g. head width, KW = 0.41, ns). The mean sizes of male bees follows exactly inversely the pattern of flower sizes: the smallest male bees were in the Plain, and the largest in Tofaha (Table 4).

Discussion

This study is interesting for two reasons. First, it demonstrates that in a situation where the environment imposes partial barriers to gene flow, there is evidence for differences in selection gradients between areas separated by only short distances. Secondly, while inferences cannot really be drawn from a sample of only four wadis, the evidence is at least consistent with the hypothesis that pollinator behaviour may be responsible for the selection gradient in maternal fitness in favour of large flowers in the Plain, an area where visit frequencies are by far the lowest of any wadi. Gene flow in natural outcrossing plant populations is brought about by both seed dispersal and pollen flow. The former component is often said to contribute little to gene flow (e.g. Rasmussen and Brodsgaard 1992): the degree to which genetic differentiation can take place is therefore normally determined largely by pollen flow, and hence in entomophilous plants by the foraging behaviour of pollinators. We are as yet unsure of the way in which seeds are dispersed in

this plant; they probably merely drop from the plants, but ants and flash floods could play a role in local and long-distance dispersal, respectively. Camels or goats eat the plants only when the plants are completely dry and the animals very hungry; while it is theoretically possible for seeds to be dispersed in the faeces of these animals, this seems very unlikely. We are currently studying both genetic differences in *Alkanna* sub-populations among wadis using randomly amplified polymorphic DNAs (RAPDs) (S. El Akkad and K. Wolf, unpublished work) and seed dispersal mechanisms. Preliminary results from RAPDs indicate that wadis are genetically distinct, and that flash flooding may be important in long-distance dispersal.

Several studies have tested whether fine-scale local genetic differentiation has taken place by testing for a distance effect on pollination success (Redmond et al. 1989; Schmitt and Gamble 1990; Widen and Widen 1990; Waser and Price 1991). Genetic differentiation may also be expressed in different floral traits over a geographic range, not necessarily the same thing as differentiation that leads to lowered success of long-distance pollinations. A variety of studies has tested for population differences in floral characters and attributed the agent of selection to pollinators. For example, Herrera (1990b) found substantial population differentiation in quantitative floral traits on both small and large geographic scales in an endemic, exclusively hawkmoth-pollinated species of violet, *Viola cazorlensis*, from southeastern Spain, but suggested that the variation indicated a lack of adaptation of the floral phenotype to its pollinator. Populations of *Dalechampia* from Mexico to Brazil have diverged morphologically in ways that have been interpreted as selection against interspecific pollination (Armbruster 1985). In the few studies where selective gradients have been measured, the evidence points to small-scale variation in these selection gradients, both spatially and temporally, as for example in *Phacelia linearis* (Eckhart 1991). In two populations of *Lobelia cardinalis* there was strong positive directional selection for flower number in both, but positive selection for height in only one population (Johnston 1991). The direction of phenotypic selection changed even over a few metres in *Impatiens pallida* (Stewart and Schoen 1987). In several of these examples the authors ascribe the agent of natural selection to insect pollinators.

In the Sinai mountains, *Alkanna orientalis* plants growing in adjacent wadis show morphological differences, and variation in the occurrence and form of selection: these populations are separated in some cases by very short distances along wadis to the Plain, and in others by the ridges between wadis. Although the ridges probably represent at least partial barriers to gene flow, we cannot rule out long-distance pollen transport since isolated patches and individual *Alkanna* plants and their *Anthophora* pollinators can be found almost up to the summits of the ridges.

Elsewhere (Willmer et al. 1994) we have shown that male *Anthophora pauperata* are territorial during most of the day, and that the females probably restrict their for-

aging to small home ranges around their nest-holes, within the territories of males. Males appear to guard the pollen and nectar resources of *Alkanna* plants in their territories for females to gather at the end of the day, after territoriality disappears (see Willmer et al. 1994). This situation makes it very likely that pollen flow is greatly restricted during the first half of the flowering season, when *Anthophora* bees are obliged (for want of an alternative) to forage from this plant. As soon as other plants begin to flower, the bees appear to switch, leaving only bombyliids, lepidoptera, and a few syrphids as the main but much less frequent foragers (Semida 1994).

We have shown experimentally here that bombyliids are able to pollinate *Alkanna*, though probably with lower efficiency. In at least one other plant, different pollinators have been shown to exert different selection pressures on floral morphology (Murcia 1990), and the differential effects of different pollinator species are becoming better known (Schemske and Horvitz 1984; Wilson and Thomson 1991). The visitors to *Alkanna* may well exert very different selection pressures too; it is unusual for the activities of two pollinators to divide the flowering season so completely.

Some but not all insect-pollinated plants have their seed set limited by pollination service (see, e.g., Bierzychudek 1981); reproductive effort through the female function in *Alkanna* may be pollen limited in the Sinai environment. We have not tested this proposition properly, since it needs a longer-term and more comprehensive experiment than we have been able to carry out to date (see Zimmerman and Pyke 1988). Two sets of experimental data from Wadi Arbaein are relevant. First, a single visit does not set as many seeds as those set by the average naturally pollinated flower – therefore multiple visits are necessary to improve seed set. Secondly, hand cross-pollinated flowers do not set any more seeds than the average for naturally pollinated flowers. Thus on average in Wadi Arbaein flowers receive enough visits to ensure all the seeds that can be pollinated do get pollinated; however, the visitation rates in Wadi Dir and on the Plain are much lower, and in these wadis we would expect seed set to be pollen limited. Behaviourally it appears to be fairly difficult for pollen-collecting female *Anthophora* to collect pollen from the flowers (Willmer et al. 1994); this is perhaps one reason why the bees switch to other plants when they come into flower.

Despite the area-restricted foraging shown by *Anthophora* bees, there is still a relationship between plant size and visitation rate. In this simple almost two-dimensional habitat, individual plants normally constitute discrete patches of flowers. Surprisingly few studies have considered the effects of patch size on visitor behaviour, but those that do (Geber 1985; Sih and Baltus 1987; Andersson 1988; Schmid-Hempel and Speiser 1988; Klinkhamer et al. 1989; Klinkhamer and de Jong 1990) always find that larger patches/plants attract more visits, but that there are fewer visits per flower and a lower proportion of the seeds are set. Sowig (1989) found different species of visitor on patches of different sizes.

We found that there were morphological differences between the male bees in the four sites. Why the smallest males (in the Plain) are found in the same sites as plants with the widest corollas and shortest styles is not clear, especially since it is the females that appear to do nearly all the pollinating (Willmer et al. 1994). It is possible that in the Plain, females may not be able to collect enough food, and may selectively underfeed their male offspring.

References

Andersson S (1988) Size-dependent pollination efficiency in *Anchusa officinalis* (Boraginaceae): causes and consequences. Oecologia 76:125–130

Armbruster WS (1985) Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). Evolution 39:733–752

Armbruster WS (1990) Estimating and testing the shapes of adaptive surfaces: the morphology and pollination of *Dalechampia* blossoms. Am Nat 135:14–31

Barnard CJ, Gilbert FS, McGregor PK (1993) Asking questions in biology. Longman, London

Bell G (1985) On the function of flowers. Proc R Soc Lond B 224:223–265

Bierzychudek P (1981) Pollinator limitation of plant reproductive effort. Am Nat 117:838–840

Crawley M (1993) GLIM for ecologists. Blackwell, Oxford

Davis PH (ed) (1978) Flora of Turkey, vol. 6. Edinburgh University Press, Edinburgh

Dewedar A (1991) The Sinai desert training centre (reports 1–4). Foreign Relations Coordination Unit (Grant 90031), Supreme Council of Universities, Egypt

Eckhart VM (1991) The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). Evol Ecol 5:370–384

Geber MA (1985) The relationship between plant size to self-pollination in *Mertensia ciliata*. Ecol 66:762–772

Herrera CM (1990a) The adaptedness of the floral phenotype in a relict endemic, hawkmoth-pollinated violet. 1. Reproductive correlates of floral variation. Biol J Linn Soc 40:263–274

Herrera CM (1990b) The adaptedness of the floral phenotype in a relict endemic, hawkmoth-pollinated violet. 2. Patterns of variation among disjunct populations. Biol J Linn Soc 40:275–291

Herrera CM (1991) Dissecting factors responsible for individual variation in plant fecundity. Ecology 72:1436–1448

Herrera CM (1993) Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. Ecol Mongr 63:251–275

Johnston MO (1991) Natural selection on floral traits in two species of *Lobelia* with different pollinators. Evolution 45:1468–1479

Klinkhamer PGL, Jong TJ de (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57:399–405

Klinkhamer PGL, Jong TJ de, Bruyn G-J de (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. Oikos 54:201–204

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Mansour RMA, Saleh NAM (1986) The flavonoids of *Alkanna orientalis*. J Nat Prod (Lloydia) 49:356

Meddis R (1984) Statistics using ranks. Blackwell, Oxford

Murcia C (1990) Effect of floral morphology and temperature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology 71:1098–1109

Polunin O, Huxley A (1972) Flowers of the Mediterranean. Chatto and Windus, London

Primack RB, Kang H (1989) Measuring fitness and natural selection in wild plant populations. Annu Rev Ecol Syst 20:367–396

Rasmussen IR, Brodsgaard B (1992) Gene flow inferred from seed dispersal and pollinator behaviour compared to DNA analysis of restriction site variation in a patchy population of *Lotus corniculatus* L. Oecologia 89:277–283

Rechinger KH (1965) Towards knowledge of the European species of the genus *Alkanna* (in German). Ann Naturhist Mus Wien 68:191–220

Redmond AM, Robbins LE, Travis J (1989) The effects of pollination distance on seed production in three populations of *Amianthium muscaetoxicum* (Liliaceae). Oecologia 79:260–264

Schemske DW, Horvitz CC (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225:519–521

Schemske DW, Horvitz CC (1989) Temporal variation in selection on a floral character. Evolution 43:461–465

Schmid-Hempel P, Speiser B (1988) Effects of inflorescence size on pollination in *Epilobium angustifolium*. Oikos 53:98–104

Schmitt JA, Gamble SE (1990) The effect of distance from the parental site on offspring performance and inbreeding depression in *Impatiens capensis*: a test of the local adaptation hypothesis. Evolution 44:2022–2030

Semida FM (1994) Insect-plant coevolution in the Sinai desert ecosystem. PhD Thesis, Suez Canal University, Ismailia, Egypt

Sih A, Baltus M-S (1987) Patch size, pollinator behaviour, and pollinator limitation in catnip. Ecology 68:1679–1690

Sowig P (1989) Effects of flowering plant's patch size on species composition of pollinator communities, foraging strategies, and resource partitioning in bumblebees (Hymenoptera, Apidae). Oecologia 78:550–558

Stanton ML, Young HJ, Ellstrand NC, Clegg JM (1991) Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. Evolution 45:268–280

Stewart SC, Schoen DJ (1987) Pattern of phenotypic variability and fecundity selection in a natural population of *Impatiens pallida*. Evolution 41:1290–1301

Täckholm V (1974) Students' Flora of Egypt, 2nd edn. Cairo University, Beirut

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1972) *Alkanna*. Flora Europaea 3:95–97

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

Weast RC (ed) (1985) CRC Handbook of Chemistry and Physics. CRC, Boca Raton

Widen B, Widen M (1990) Pollen limitation and distance-dependent fecundity in females of the clonal dioecious herb *Glechoma hederacea* (Lamiaceae). Oecologia 83:191–196

Willmer PG, Gilbert FS, Ghazoul J, Semida F, Zalat SM (1994) A novel form of territoriality: daily paternal investment in an anthophorid bee. Anim Behav 46:535–549

Wilson P, Thomson JD (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. Ecology 72:1503–1507

Young HJ, Stanton ML (1990) Influences of floral variation on pollen removal and seed production in wild radish. Ecology 71: 536–547

Zimmerman M, Pyke GH (1988) Reproduction in *Polemonium*: assessing the factors limiting seed set. Am Nat 131:723–738