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Experimental studies of pollen carryover: effects of floral variability in *Ipomopsis aggregata*

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Summary. In the montane herb *Ipomopsis aggregata*, size and placement of stamens and pistils vary substantially among flowers within plants, among nearby plants, and among groups of plants separated by 50–100 m. We trained captive hummingbirds to feed from flowers of this species in a flight cage, and explored the effects of different degrees of floral variability on carryover of fluorescent dyes that act as pollen mimics. We found that the slopes of linear dye carryover functions generally became more shallow as floral variability increased; this led to substantially longer carryover in the treatment with greatest variability. On the other hand, total amounts of dye transferred did not appear to be sensitive to the degree of variability. Floral variability may have a subtle but important effect on plant fitness by influencing the distance of pollen transfer.

"It should be remembered that systematists are far from pleased at finding variability in important characters..." – Darwin 1859, p. 45.

In the second chapter of *The Origin of Species*, Darwin (1859) noted the reluctance of his biological contemporaries to acknowledge that members of a single species could differ in morphology. Until fairly recently, morphological variability has been studied mostly on a geographical or interpopulational scale, with a primary goal being to elucidate phylogenetic relationships. In the case of higher plants, for example, reproductive structures such as flowers, fruits, and seeds are frequently used as diagnostic taxonomic characters, and are taken *ipso facto* as fixed within a taxon such as a species, subspecies, or ecotype (Grant 1949; Stebbins 1950). Fine-scale variation in these traits is thus effectively glossed over in many systematic treatments, whereas geographic variation evokes considerable debate about the taxonomic status of each variant (for an example especially pertinent here see Wherry 1961, Grant and Grant 1965). Except for studies of striking but relatively rare polymorphisms in flower color, floral form (e.g., within heterostylous species), or similar traits, much less effort has been spent on documenting the degree and kind of morphological variability in reproductive structures within plant populations, and on considering whether it has immediate effects on the fitness of individuals.

The fitness consequences of some types of variability may be subtle. The example we consider here involves intrapopulational variability in placement of the sexual parts of flowers, and the ways this might influence the amount and distance of pollen transfer among individuals. Our interest stems in large part from computer models in which Lertzman (1981; see also Lertzman and Gass 1983) explicitly simulated hummingbird pollination of *Castilleja miniata*. Lertzman found that pollen carryover – the extent to which pollen from one flower is picked up and transferred beyond the next flower visited – varied in his model system as a function of variability in exsertion and lateral placement of anthers and stigmas. The probability of successful pollen pickup and transfer seemed to be highest with a small degree of anther and stigma variation. On the other hand, the carryover distance in cases where at least some pollen was successfully transferred increased monotonically with increasing floral variability (see also Waser 1983a). We will discuss the mechanistic bases for these trends later, insofar as they are understood.

Here we extend our earlier studies of carryover in the montane perennial monocarpic herb *Ipomopsis aggregata* (Waser and Price 1982; Price and Waser 1982). By manipulating the amount of variability in anther and stigma placement among flowers and observing the effect on transfer of powdered dyes by hummingbirds, we were able to explore the general prediction from Lertzman's models that variability will affect carryover, as well as the specific predictions that as the degree of variability increases, distance of carryover will increase, while overall probability of pollen transfer will decrease.

Methods

(1) Measurement of natural floral variability

Around the Rocky Mountain Biological Laboratory (RMBL) in western Colorado, *Ipomopsis aggregata* exhibits floral traits considered "typical" of the species (Wherry 1961; Grant and Grant 1965; D. Wilken, pers. comm.), including moderate corolla length and bright scarlet coloration. There is substantial morphological variation among flowers, however. Variable traits include corolla length and width, as well as length of the style and consequent degree of insertion or exsertion of the stigma. Anther placement also varies: filaments differ in length, so that some flowers have anthers exserted and others inserted relative to corolla and stigma. In addition, each flower has 2–3 adjacent fila-

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ments (of 5 total) that are up to 3 mm longer than the others, which makes some anthers more strongly exserted. Finally, there is variation in the position of these most strongly exserted anthers when flowers on undisturbed plants are viewed from the front. We will refer to this henceforth as the "radial clock position" of anthers, since it can be summarized by analogy to position on a clock face where 12 o'clock is straight up.

During the summer of 1982 we studied 3 populations of *I. aggregata* at the RMBL separated from one another by 50–100 m. Taking 6 flowers from each of 5 near-neighbor plants (each growing <1 m from the last) in each population, we measured corolla lengths and widths, style lengths, and distances from the base of the corolla to the proximal tip of the shortest and distal tip of the longest anther. These last two measurements defined the boundaries of the "anther zone" (Fig. 1). In 1982 we also characterized the radial clock position of the most strongly exserted anther in 10 flowers from each of 20 plants in one of our study populations.

(2) Collection of flowers for carryover experiments

Because there seemed to be two major modes of morphological variation in *I. aggregata* around the RMBL – variation in length of style on the one hand, and radial position of exserted anthers on the other – we designed treatments in which we manipulated variability in style length among recipient flowers independently of variability in anther position. In each case we produced three degrees of variability – minimal, natural, and maximal – so that there were 3 x 3 = 9 distinct treatments (Table 1).

Whenever possible, we chose flowers for carryover treatments so as to mimic natural hummingbird visitation. Since these major pollinators of *I. aggregata* visit an average of 6 flowers per plant before flying to a neighboring plant (Waser 1982), we took 6 flowers from one plant, then 6 from a near neighbor <1 m away (except in treatments 7–9; see below), and so on until enough flowers had been collected to run an individual experimental replicate. To achieve minimal stylar variation, we chose neighbors with the same degree of stylar exsertion – either all "long" (i.e. stigma exserted beyond the anther zone), all "short" (i.e. stigma inserted below the anther zone), or all "medium" (i.e., stigma placed within the anther zone). To achieve natural stylar variation, we chose neighbors at random. To achieve maximal stylar variation, we alternated long-, medium-, and short-styled plants. Because it was difficult to find near neighbors with such different degrees of stylar exsertion, we usually had to pick plants for maximal stylar variation treatments that were separated by at least 5 m. For a given set of treatments run on the same day (see below), we controlled sex ratio by choosing as closely as possible a constant proportion of male-phase flowers (between 11% and 59%) and interspersing them evenly with female-phase flowers.

To achieve minimal variation in anther radial position we held all flowers with the most strongly exserted anther at the 6 o'clock position. To achieve natural variation we held flowers in the original orientation they had exhibited when we had picked them from undisturbed plants. To achieve maximal variation we alternated flowers with the most strongly exserted anther at 6, 9, 12, and 3 o'clock positions.

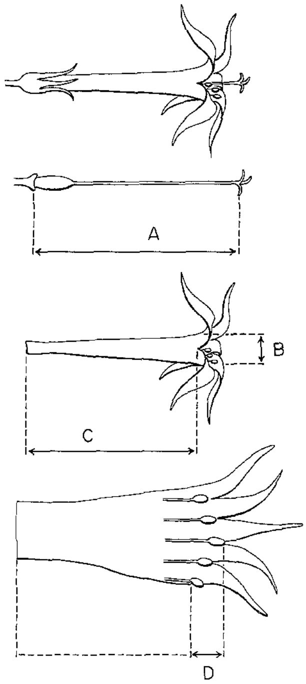

Fig. 1. Diagrammatic representations of an *Ipomopsis aggregata* flower (top) showing the measurements used to characterize floral morphology. *A* style length; *B* corolla width; *C* corolla length; *D* anther zone

Table 1. Summary of floral variability treatments

Treatment	Variation in	
	Style length	Anther position
1	minimal	minimal
2	minimal	natural
3	minimal	maximal
4	natural	minimal
5	natural	natural
6	natural	maximal
7	maximal	minimal
8	maximal	natural
9	maximal	maximal

We measured flowers actually used in 1983 replicates of treatments 1–6, and flowers collected in 1983 in a fashion that simulated the way we had collected flowers for treatments 7–9 in 1982, to confirm that the degree of variability in style length actually did increase as intended among treatments. This was the case: mean pairwise differences in style length between adjacent flowers in carryover sequences increased from 1.45 mm to 1.84 mm to 2.73 mm in treatments 1–3, 4–6, and 7–9, respectively; a one-way ANOVA yields F = 5.26, d.f. = 2, 398; $P < 0.01$ for these differences. Analogous pairwise differences in corolla length increased from 1.58 mm to 1.77 mm to 1.90 mm; ANOVA yields F = 2.68; d.f. = 2, 1243; $0.05 < P < 0.1$. Thus the degree of variation in corolla length also differed among treatments, but in a way that was concordant with variation in style length.

(3) Design of carryover experiments

Our protocol followed that of Waser and Price (1982) and Price and Waser (1982). We captured male or female rufous (*Selasphorus rufus*) hummingbirds at sugar-water feeders, introduced them into an outdoor flight cage (Hansen

Weather Port Corp., Gunnison, CO), and trained them to visit individual flowers held in the hand in a standard fashion. We then presented them with experimental sequences of 30–37 flowers in which there were different degrees of floral variability as described above. The first flower in each sequence was a male-phase donor whose anthers had been dusted carefully with a colored dye powder (Hercules Inc., Richmond, CA) that has carryover properties similar to those of pollen (Waser and Price 1982). Using a dissecting microscope at 50X, we counted dye particles adhering to receptive stigma surfaces of each female-phase recipient flower. Because amounts of dye initially picked up varied among experimental replicates, we adjusted raw counts by dividing them by the largest value counted on any stigma in that replicate. In 1982 we repeated treatments 4 through 9 11–12 times each, and in 1983 repeated treatments 1 through 6 12 times each, for a total of 142 experimental replicates.

In addition to the potential effect of floral variability, behavioral and morphological variability of the pollinators themselves are likely to influence carryover, as Lertzman and Gass (1983) have noted. At this point in our study we wished to concentrate on floral variability alone, and to control as much as possible for variation between individual pollinators. Thus we used only one species of hummingbird in experiments (there is a second common species at the RMBL). We also always ran complete sets of 6 experimental treatments as a unit, using the same individual bird, and did this as quickly as possible. Time permitting, we then repeated the procedure, with the stipulation that we never ran part of a set of treatments on one day and the rest on another. Over 2 years we used a total of 4 birds (2 males and 2 females); each bird was used for between 4 and 8 replicate sets of experimental treatments. We cleaned birds of residual dye between treatments by allowing them to visit 40–50 flowers that had been freshly picked in the field; we also alternated colors of dye used between successive treatments.

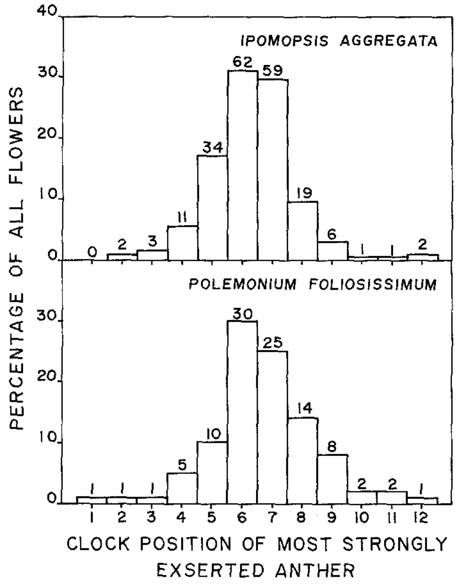

Fig. 2. Frequency distributions of radial position of the most strongly exserted anther from flowers on several neighboring plants in populations of *Ipomopsis aggregata* (top) and *Polemonium foliosissimum* (bottom). Absolute numbers of flowers in each category are given above histograms. The two frequency distributions are statistically homogeneous (G = 9.35, df = 7, $0.5 > P > 0.1$)

Results

(1) Natural floral variability

In populations at the RMBL there is substantial within- and between-plant variation in radial position of the most strongly exserted anther. While positions around 6 o'clock predominate, essentially all are possible (Fig. 2, top). A one-way ANOVA indicates that variation among plants is highly significant (F = 3.03; df = 19, 180; $P = 0.0001$).

There is also variation within plants, between plants within populations, and between populations 50–100 m apart in aspects of corolla, stamen, and pistil size (Table 2, left). Coefficients of variation for different populations and traits range from 5.5% to 23.2%, with most values near 10%. MANOVA shows that there is significant variation in the entire suite of traits taken together, both among plants within populations (relative to variation among flowers; Wilks' lambda = 0.034; F = 2.98; df = 48,102; $P = 0.0001$) and among populations (relative to variation among plants; Wilks' lambda = 0.058; F = 7.12; df = 8,18; $P = 0.0003$). Results of ANOVA (Table 2, right) indicate

Table 2. Variation within and among plants and populations in floral traits other than radial position of the most strongly exserted anther. Values are in mm and are expressed as $\bar{X} \pm 1$ se (N), followed on the next line by CV. "Anther Length" refers to the distance from the base of the corolla to the midpoint of the anther zone. The right-hand part of the table shows F and P values (the latter in parentheses) from nested ANOVAs with individual traits, for variation among populations ("among") and among plants within populations ("withing")

Morphological trait	Population			Source of variation	
	Coal pile	Tincup	Murray	Among	Within
Style length	24.0 ± 1.48 (14) 23.2%	28.2 ± 0.76 (17) 11.0%	22.9 ± 0.42 (12) 6.3%	37.18 (0.0001)	3.85 (0.0015)
Corolla length	25.7 ± 0.25 (30) 5.5%	28.4 ± 0.30 (30) 5.8%	24.8 ± 0.33 (30) 7.1%	17.51 (0.0001)	1.02 (0.457)
Corolla width	3.9 ± 0.07 (30) 10.7%	4.6 ± 0.09 (30) 10.9%	3.7 ± 0.06 (30) 9.2%	15.45 (0.0001)	3.13 (0.0059)
Anther length	24.1 ± 0.24 (30) 5.6%	27.8 ± 0.28 (30) 5.6%	23.5 ± 0.29 (30) 6.8%	24.38 (0.0001)	0.50 (0.899)

Table 3. Regressions of mean adjusted dye deposition on flower position in a visitation sequence. Linear model ("Lin") is $y=a+bx$; exponential model ("Exp") is $y=ae^{bx}$

Treatment	Model	Regression parameters			ANOVA				
		r^2	a	b	Source of variation	df	MS	F	P
#1	Lin	0.51	0.38	−0.011	Regression	1	0.451	34.37	≪0.001
					Deviations	33	0.013		
	Exp	0.69	0.43	−0.068	Regression	1	16.634	71.92	≪0.001
					Deviations	33	0.231		
#2	Lin	0.60	0.40	−0.011	Regression	1	0.440	51.82	≪0.001
					Deviations	34	0.008		
	Exp	0.52	0.43	−0.055	Regression	1	11.747	37.08	≪0.001
					Deviations	34	0.317		
#3	Lin	0.41	0.36	−0.010	Regression	1	0.427	24.54	≪0.001
					Deviations	35	0.017		
	Exp	0.60	0.39	−0.065	Regression	1	17.915	51.42	≪0.001
					Deviations	35	0.348		
#4	Lin	0.60	0.36	−0.010	Regression	1	0.446	52.12	≪0.001
					Deviations	35	0.009		
	Exp	0.52	0.35	−0.053	Regression	1	11.640	42.40	≪0.001
					Deviations	35	0.303		
#5	Lin	0.70	0.38	−0.009	Regression	1	0.373	80.85	≪0.001
					Deviations	35	0.005		
	Exp	0.67	0.45	−0.054	Regression	1	12.239	70.90	≪0.001
					Deviations	35	0.173		
#6	Lin	0.72	0.40	−0.012	Regression	1	0.606	88.02	≪0.001
					Deviations	35	0.007		
	Exp	0.75	0.53	−0.080	Regression	1	27.060	102.19	≪0.001
					Deviations	35	0.265		
#7	Lin	0.51	0.32	−0.009	Regression	1	0.328	34.61	≪0.001
					Deviations	34	0.009		
	Exp	0.48	0.30	−0.059	Regression	1	13.333	32.00	≪0.001
					Deviations	34	0.417		
#8	Lin	0.48	0.30	−0.007	Regression	1	0.228	32.02	≪0.001
					Deviations	34	0.007		
	Exp	0.46	0.33	−0.059	Regression	1	13.378	29.53	≪0.001
					Deviations	34	0.453		
#9	Lin	0.14	0.30	−0.004	Regression	1	0.072	5.45	<0.05
					Deviations	34	0.013		
	Exp	0.12	0.27	−0.021	Regression	1	1.656	4.70	<0.05
					Deviations	34	0.352		

significant interpopulational variation in each trait taken by itself, and significant intrapopulational variation in corolla width and especially in style length.

(2) Effects of variability on dye carryover

For all 9 treatments mean adjusted values for dye deposition declined as a function of flower position in a carryover sequence. Both linear and exponential fits were highly significant, but linear fits were better than exponential in 6 of 9 cases, judging from r^2 and F values (Table 3). In what follows, therefore, we will use linear regressions to summarize the effects of different treatments on dye carryover. The regressions for treatments 4, 5, and 6 were run on means pooled across replicates from 1982 and 1983, since slopes of linear fits from the 2 years were homogeneous (in all 3 cases $F<3.9$; $df=1,69$; $P>0.05$; Sokal and Rohlf 1981, p 500 ff).

There was significant overall heterogeneity among regression slopes for the 9 treatments ($F=2.06$; $df=8,309$; $P<0.05$). Examination of 95% confidence intervals of slopes (Fig. 3) reveals that this is explained mainly by differences between treatments 9 and 1 and 9 and 6. While most pairwise comparisons yielded homogeneous regression slopes, there was a consistent tendency for slopes to become more shallow as the degree of floral variability increased from treatment to treatment. Of 12 pairwise differences between slopes of adjacent treatments in Table 4, 10 went in this direction ($P=0.02$, sign test); the two exceptions were entirely due to treatment 6, which has a relatively

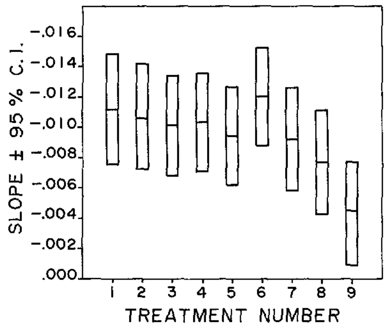

Fig. 3. Linear carryover slopes and 95% confidence limits for the 9 treatments, from an unplanned comparison of regressions (Sokal and Rohlf 1981, p 507)

Table 4. Slopes of linear carryover fits for the 9 treatments and expected rank of flower reached in a visitation sequence (mean carryover; in parentheses). A more shallow slope implies longer carryover, if total amount of dye transferred in a replicate does not vary among treatments. Carryover was predicted to grow longer as degree of floral variability increased (from top to bottom and left to right of Table)

	Anther placement variability		
	Minimal	Natural	Maximal
Style length variability			
Minimal	−0.0112 (11.4)	−0.0107 (12.6)	−0.0101 (11.8)
Natural	−0.0103 (11.8)	−0.0094 (13.3)	−0.0120 (11.2)
Maximal	−0.0092 (11.5)	−0.0072 (12.9)	−0.0043 (22.9)

steep slope (Fig. 3, Table 4). If one considers only the extreme treatments (those in the 4 corners of the table), all comparisons were in this direction ($P=0.06$).

While slopes consistently became more shallow with increasing floral variability, this did not always lead to longer carryover. In Table 4 as a whole, 7 of 12 pairwise comparisons between treatments were in the direction of increasing mean carryover with increasing floral variability ($P=0.39$); most means were around the 12th flower in a visitation sequence (the mean is one third of the x-intercept). On the other hand, all 4 comparisons among the most extreme treatments (those in the corners of the table) went in the direction of increasing carryover ($P=0.06$), as did the 2 comparisons along the diagonal from minimal to maximal variation. Pairwise differences were small except in the case of treatment 9, which produced much longer carryover than any of the other 8 treatments.

In contrast to distance of carryover, there was no tendency for amounts of dye deposited on stigmas of female-phase flowers to differ among treatments. Mean unadjusted numbers of dye particles on stigmas (calculated by averaging across flowers in each carryover replicate and then averaging across all replicates of a treatment) ranged from 5.0 to 10.6 and clustered around 8, and only 5 of 12 pairwise comparisons of treatments went in the direction of decreasing mean transfer with increasing floral variability ($P=0.61$). A one-way ANOVA using means from each individual replicate also indicates that there are no significant differences across treatments ($F=0.95$; df$=8$, 133; $P=0.48$). An examination restricted to the extreme treatments gives the same result: only 2 of 4 pairwise comparisons went in the direction of decreasing dye transfer with increasing floral variability ($P=0.90$).

Discussion

The sort of phenotypic floral variability we have documented within *I. aggregata* populations is not at all exceptional, as any field ecologist working with such systems would likely affirm. To our knowledge, however, published reports of such patterns remain rare. Ellstrand (1983) recently described variability in numbers of floral parts within and among *I. aggregata* plants in some of the same populations we studied, and Vickery (1978 and personal communication) has amassed extensive measurements of floral traits within small populations of *Mimulus guttatus*. In the latter case, plants were grown in a common greenhouse environment so that genetic variation could be isolated; such an approach is especially rare. Neither of these studies presents results directly comparable to ours, however. In the course of designing his computer simulations, Lertzman (1981) did make measurements very similar to ours on *Castilleja miniata* flowers. He reported coefficients of variation for flower length and lateral placement of sexual parts ranging from 29% to 180%, well above the values we observed (Table 2). The difference between Lertzman's results and ours may be due to his collecting flowers "at random" within a meadow whereas we collected systematically from near neighbors, and to his inclusion of several flower age classes, which correspond strongly to size classes in his species but not in ours.

Floral variability in *I. aggregata* indeed seems to influence carryover, as Lertzman's (1981) models predict. We did not observe all the effects expected from his models, however. The slopes of linear carryover functions did grow consistently more shallow with increasing variability, and this translated into a substantial increase in carryover distance in the most extreme treatment at least. On the other hand, we detected no effect of variability on amounts of dye tranferred in carryover sequences. Thus our results lend empirical support to one major prediction from Lertzman's models, but not to another.

In almost all cases, any effects we uncovered were slight, especially compared to to those seen in Lertzman's simulations. One reasonable explanation has to do with the degree of variability we achieved in our experimental manipulations. Our minimal and maximal variation treatments were certainly as extreme as feasible, and must have produced coefficients of variation smaller and larger, respectively, than those found with natural variation. Still, coefficients of variation were nowhere near the range of 20% to 400% Lertzman chose for his models, based on his measurements of *C. miniata*! Another thing that may have influenced Lertzman's results was his assumption that pollen from successively-visited flowers is deposited in a series of discrete layers on the pollinator, with a stigma only removing the upper layer in the part of the pool of pollen on the pollinator that it contacts.

The features of Lertzman's models seem to lead to his results for the following mechanistic reasons. If there is no variability all flowers deposit pollen in completely overlapping layers, and all stigmas receive pollen only from the immediately previous flower. As variability increases, carryover distance does also, because pollen from a given flower is not immediately buried and once buried has a chance to "resurface". If Lertzman had assumed that pollen was mixed rather than layered in the pollen pool, the mechanics of pollen transfer and the influence of variability on carryover distance might have been different; it is not clear that layering rather than mixing is occurring in the *I. aggregata* – hummingbird system (Price and Waser 1982). The decrease in successful pollen transfer with increasing variability in the simulations seems to be explained, in turn, by increasing failure of stigmas to contact the pollen pool at all, and by the pollen contribution from some flowers being placed in peripheral parts of the pool that are seldom contacted. Even our maximal variation treatments were not so extreme as to make failure of dye transfer onto stigmas a common event, and indeed we detected no overall differences in total dye transfer. On the other hand, flower-to-flower variation in amounts transferred did appear to be more pronounced in treatment 9 than in others, as evidenced by the low values of F and r^2 for this treatment (Table 3).

If the range of variability that we produced experimentally is taken as an estimate of what might possibly occur in natural situations of pollen carryover, can any reproductive consequences be expected? Our results suggest that the degree of variability will not influence fitness through any effect on the amounts of pollen transferred to or away from the flowers of a plant. On the other hand, effects on the distance of pollen carryover, i.e., on the mean and maximum number of flowers a pollen grain is carried away from its source, may well influence fitness. We have experimental evidence for *I. aggregata* and another species that mean seed set and seedling survival differ with surprisingly small changes in the physical separation of plants between which pollen transfer occurs, and that a short outcrossing distance is optimal (Price and Waser 1979; Waser and Price 1983 and references therein, Waser 1983b and unpublished; see also Lertzman 1981).

The mean carryover distance is around the 12th flower in all but one of our treatments; in that treatment it is the 23rd flower (Table 4). Contrary to first impressions, this is not "long carryover": assuming that hummingbirds visit 6 flowers per plant, the average dye particle (and presumably pollen grain) will reach only the second to fourth plant – depending on the experimental treatment – beyond the one that donates it. With interplant spacing of 50 cm being representative in *I. aggregata* populations around the RMBL (e.g., Waser 1982), this translates into a linear distance of 1–2 m at most, even assuming that hummingbirds move in a straight line from plant to plant. Allowing for directionality changes in successive interplant flights, the mean dispersal distance is even less. By our experimental measures the optimal outcrossing distance for this species appears to be roughly 10 m. Earlier studies also suggested a discrepancy between optimal distance and the mean and median distances of potential gene flow via pollen (Waser and Price 1983).

Given that expected pollen transfer distances fall short of some optimum, selection for increased variability among flowers within a plant or frequency-dependent selection for increased variability among neighboring plants might operate insofar as variability enhances carryover distance. Selection would act as long as there was an immediate fitness advantage, regardless of whether the advantage eventually disappeared after genetic structure of the population and optimal outcrossing distance equilibrated with the altered pollen flow regime. Waddington (1983) has argued that changes in pollen flow distance will change the optimal distance to the same extent so that the two will never converge. The verbal and graphical arguments he musters are not sufficient to support this conclusion, however, because the effects of changes in pollen flow on patterns of genetic correlation with distance (and thus on the location of the optimal outcrossing distance) are not necessarily the simple ones he assumes (see Kimura and Weiss 1964; Crow and Kimura 1970, chapter 3).

Is there any evidence that the degree of *I. aggregata* floral variability that would actually occur in natural carryover sequences is in part an adaptation to increase pollen transfer distances? It is difficult to imagine strong sorts of evidence that can be brought to bear on a question such as this (see Maynard Smith 1978; Waser 1983a), but we can think of several sorts of weak evidence. First, we have measured radial position of the most strongly exserted anther in flowers of *Polemonium foliosissimum* at the RMBL, using plants near the *I. aggregata* plants on which similar measurements were made. The degree of variation in this trait is statistically indistinguishable for the two species (Figure 2), although they are not especially close relatives (being confamilials but not congeners) and have different flower shapes and major pollinators (bumblebees in the case of *P. foliosissimum*). This suggests that variability, in this trait at least, is either not an adaptation or is a convergent adaptation in both species. Similarly, we have noticed from Table 4 that the carryover effects of natural variation (treatment 5) are more similar to those of minimal variation (treatment 1) than to those of maximal variation (treatment 9). The opposite might be expected if selection favored variability and was not otherwise constrained, since treatment 9 produces by far the longest carryover. We do not consider either of these observations telling evidence against the hypothesis that observed natural variation in *I. aggregata* is the most extreme possible that selection can produce. Instead, the observations mostly serve to emphasize that, while it is difficult to demonstrate that a trait such as floral variability has an effect on fitness at present, it is even more difficult to deal with questions of the adaptive nature and evolutionary history of the trait.

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