

Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumblebee bodies

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Selection on flowers has often been viewed as being particularly strict, constant, and responsible for species differences. *Impatiens pallida* and *I. capensis* flowers fit snugly around bees, leading one to expect a close relationship between floral morphology and pollination success. My studies on the amount of pollen removed from androecia and deposited on stigmas in single visits by bumblebees did not confirm this supposition. Trimming off parts of the floral vestibule with scissors and gluing in pleats had very little effect on the amount of pollen that bees moved. In reciprocal transfer experiments, flowers from different populations sometimes differed in the amount of pollen moved, but when the two species were compared in sympatry, pollen removal and deposition differed hardly at all. A comparison of the relationship between pollen movement and floral morphology among 15 populations showed that, although there was great heterogeneity in the amount of pollen moved, the observed differences were independent of floral morphology. None of this supports a belief in strong selection that fine-tunes the mechanical fit between bee and flower; selection for visitation success based on pollinator behaviour may have a much stronger influence on floral characters.

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ADDITIONAL KEY WORDS:—*Bombus* – pollen removal – pollen deposition – floral morphology.

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INTRODUCTION

Flowers are orderly objects. Compared to shoots, roots, or leaves, they are relatively invariant within species and are relatively unaffected by environmental and developmental circumstances. Also, the morphology of flowers is often ornate, with each part just in its place and with many colour patterns, spurs, pockets, and flanges that supposedly attract pollinators and then channel them past the anthers and stigmas where pollen is dispatched and received with what has often been thought to be exacting precision (Müller, 1883; Straw, 1956; Percival & Morgan, 1965; Macior, 1967; Beattie, 1971a; Brantjes, 1982; Wolf & Stiles, 1989; Armbruster *et al.*, 1994). There is no end to the number of cases in which naturalists have remarked on how the characteristics of the flower act to place pollen on the nape of a bird's neck (Pickens, 1927), or in a safe-site between a bee's legs (Macior, 1982), or on a particular place on a moth that is required to probe the full length of its proboscis into the flower's deeply recessed nectary (Nilsson, 1988), and the many details of floral design and ornamentation generally seem exceedingly organized. Stebbins (1974: 50) made the point well and with a hint of an explanation:

An important difference between adaptations for survival in higher plants and adaptations for their various reproductive functions is that the latter require much higher levels of integration between the different organs, in fact, levels that are quite comparable to those found in animals. For adaptation to cross-pollination by animals with specialized habits, such as bees, butterflies, moths, birds, and bats, the flower must become a highly integrated structure, with all of its parts precisely adjusted to one another.

In counterpoint to the complex constancy of flowers within species, they also often display more than their share of features that distinguish species and higher taxa. This point will be obvious to anyone with experience in plant identification, but the evidence for it is not well tabulated. Grant's 1949 paper was a start. He tallied the number of taxonomically useful floral and vegetative characters in animal- and abiotically-pollinated plants, and found that a higher proportion of informative characters were floral when pollination was via animals. This might be expected from the habits of taxonomists or from the complexity of zoophilous flowers, but the suggestion that Grant made was that in animal-pollinated plants floral morphology functions as a reproductive isolating mechanism, and as such is constrained within species but driven (or at least free) to vary among species. At the time, it was fashionable to think of isolating mechanisms as adaptations to avoid hybridization (Dobzhansky, 1937). Now such a presumption would be viewed with skepticism (Futuyma, 1986: 242–244; Grant, 1994), but even without reinforcement, the possibility that flowers are under particularly constant and strong selection within species but not constrained between species seems like a reasonable hypothesis for explaining a major feature of plant diversity. At any rate, many authors have intimated that there is something special about flowers that has facilitated evolution in their characters during the divergence of species (Davis & Heywood, 1963: 433–441; Takhtajan, 1969: 40; Ornduff, 1969; Thompson, 1982: 121; West-Eberhard, 1983; Crepet, 1984).

Floral characters can function with respect to pollinators either by affecting their behaviour, for instance attracting them with showy petals and sweet nectar, or by affecting the physical contact between the animal and the anthers or stigma, such as by forcing it to crawl into a gullet. This distinction goes back at least to Kerner (1895) who treated pollination under a series of section headings 'Allurements of animals with a view to the dispersion of pollen', 'Taking up of pollen by insects', and 'Deposition of pollen'. The distinction was also evident when Grant wrote of 'ethological' versus 'mechanical' isolating mechanisms. Most experimental studies of the adaptive nature of floral traits have focused on how they influence animal behaviour and visitation success. Plateau's studies at the turn of the century and Clements & Long's 1923 monograph provided abundant data on how experimental alternations of floral morphology can greatly reduce visitation rates. There have been a number of more recent studies that have suggested that animals are often unlikely to respond properly to an altered signal (see Discussion). While the suspicion has grown that flowers are under constant selection through pollinator visitation rate, there has only very recently been more than anecdotal evidence on how they function mechanically to dispatch and receive pollen (Harder & Thomson, 1989; Galen & Stanton, 1989; Young & Stanton, 1990; Murcia, 1990; Wilson & Thomson, 1991; Mitchell & Waser, 1992; Harder & Barrett, 1993).

Here I consider how the details of flower morphology do or do not influence how much pollen is removed from the androecium in a visit and how much pollen is deposited on the stigma in a visit. Unfortunately, I do not have data on the separate issue of how these traits might be related to the probability of an insect visiting the flower in the first place. Studying pollen removal and deposition in a visit does impose a definite limitation on my work: pollination success is a far cry from fitness (Stanton *et al.*, 1992). However, unlike seed siring and seed setting, studying pollen removal and deposition facilitates an understanding of the mechanistic basis for differential pollination success ('performance' *sensu* Arnold, 1983). It is through removal and deposition that bees directly influence fitness. In addition, I was able to do far more experiments by counting pollen grains than if I had tracked paternity and maternity, and perhaps even more important, my statistical resolution on the determinants of pollination success was bound to be much better than on the pollination factors that determine, say, lifetime reproductive success (Grafen, 1988). I was, therefore, maximizing my ability to tease apart the ways in which the pollination process can give rise to selection, and my evidence does not support the view that floral morphology is strenuously selected for mechanical fit in determining how much pollen is removed and how much is deposited.

MATERIALS

The two jewelweed species of eastern North America, *Impatiens pallida* and *Impatiens capensis*, have flowers that are pollinated by bumblebees. *I. pallida* is yellow, and *I. capensis* is orange, and this colour difference is probably the sole reason that the former is regarded as a bee flower and the latter a hummingbird flower (Wood, 1975; also see Bertin, 1982). In fact,

hummingbirds visit both species and are good pollinators of both, but at least at the sites where I have done my work they account for only a tiny proportion of the visits. Bumblebees are the principal pollinators (*sensu* Stebbins, 1974), and the architecture of the flowers is strongly suggestive of adaptation specifically to bumblebees (Rust, 1977a). The flowers are protandrous. When they open, they are in male phase, which lasts for a little over 1 day. Then the gynoecium swells, pushes away the androecium, and exposes its receptive stigma. Female phase lasts from a few hours to almost 1 day before the perianth falls away, leaving the fruit to mature. The perianth consists of a banner petal that the bees do not normally touch, two porch petals (each actually a fused pair) that the bees stand on and that also provide much of the advertisement surface, two small bud-covering sepals, and one large vestibular sepal that the bees crawl into and that ends in a recurved nectar spur. The nectar spur is about 6 mm long in *I. pallida* and about 8 mm long in *I. capensis*. It cannot be emptied by short-tongued bees nearly as thoroughly as by bumblebees (P. Wilson unpubl.). *I. pallida* nectar contains fewer amino acids than the nectar of *I. capensis* (Rust, 1977a). *I. capensis* flowers have been shown by Marden (1984) to secrete 0.28 μl of nectar per hour with a sugar concentration of 41%: they are a very rich resource. The porch petals and vestibule have a size that fits tightly around a bumblebee to a greater or lesser extent depending on the individual plant and the species of bee (Wilson & Thomson, in press). The androecium (in male phase) or the stigma (in female phase) is positioned inside the flower pointing down from the roof of the vestibule, and pollen is placed onto and received from the back of the bee. A bee that is visiting jewelweeds develops a white stripe of pollen down her thorax between the wings.

Jewelweeds have been the subject of many previous studies in evolutionary ecology. Their natural history is well described by Schemske (1978). Local populations are remarkably differentiated in morphology and in the plant's ability to grow and reproduce (Schemske, 1984; Schmitt & Gamble, 1990). An allozyme survey of 11 populations of *Impatiens capensis* has revealed a highly heterogeneous genetic structure, with some populations having very high fixation indices and others very low (Knight & Waller, 1987). Within one large population of *I. pallida*, Stewart & Schoen (1987) found strong and heterogeneous phenotypic selection on plant-size and phenology characters. In a second study involving both species, Brassard & Schoen (1990) found that the selective heterogeneity was not maintained when the habitat was physically homogeneous. Both of the *Impatiens* species facultatively produce cleistogamous rather than chasmogamous flowers when they are experiencing drought, deep shade or harsh competition; seeds from the two types of flowers differ in vigour due in part to the effects of inbreeding versus outcrossing (McCall *et al.*, 1989, and many citations therein). Mature seeds are catapulted away from the mother plant a short distance (51 cm on average), and secondary dispersal by water and rodents can be much greater (Schmitt *et al.*, 1985, and personal communication). The flowers have distinct male and female phases, and the male phase lasts longer and produces more nectar than the female phase. Bumblebees respond to this by undervisiting female-phase flowers (Bell *et al.*, 1984).

Impatiens pallida and *I. capensis* are vegetatively indistinguishable but

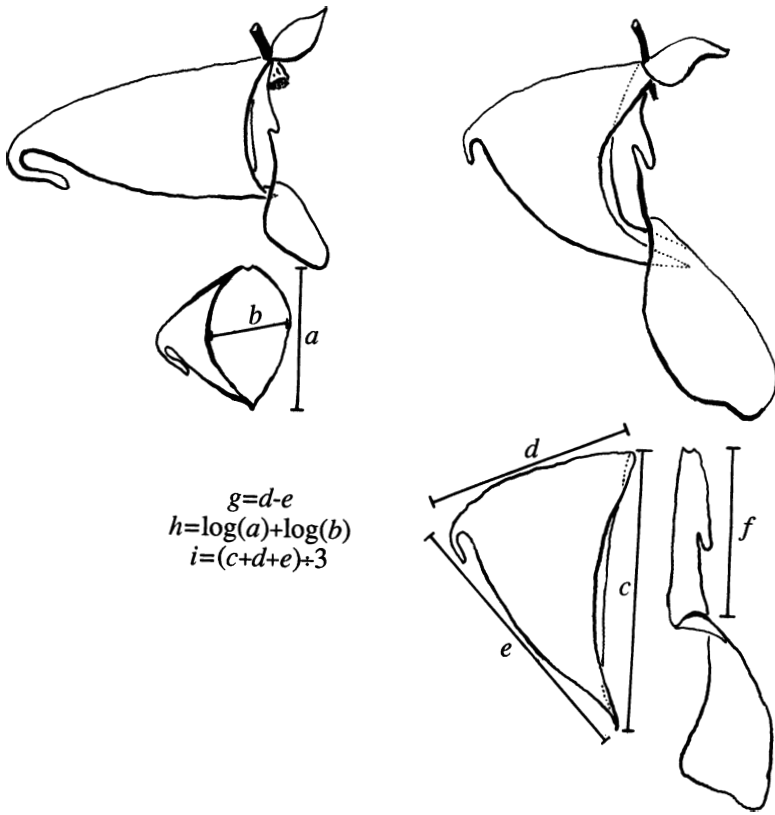


Figure 1. Floral measurements of *Impatiens capensis* (left in male phase) and *I. pallida* (right in female phase). The dimensions c and f were taken after gently flattening the sepal and petal, respectively. The quantities g , h , and i were calculated from the other dimensions.

reproductively intersterile (Randall & Hilu, 1990). The flowers (Fig. 1) differ in having, respectively, a yellow versus an orange perianth, larger versus smaller porch petals, a smaller versus a larger spur, and a shorter more tapering vestibule versus one that is more cylindrical. Wherever possible I have done my studies in parallel on both species. The original rationale for this was to ask the question: once two lineages have diverged somewhat in floral morphology, does that change the way selection acts upon those flowers? However, I found (1) that floral morphology as it naturally varies explained almost no variance in the amount of pollen moved ('non-self-determination'), (2) that the direction of the effect, when evident, did not follow *a priori* expectations based on mechanical fit ('unpredictability'), and (3) that pollen movement was much more dependent on external factors such as site and year than on the characteristics of the flowers ('contextuality').

GENERAL METHODS

My data consist of perianth measurements and pollen counts of flowers visited by naturally foraging bumblebees. I controlled the flowers' stage of maturity and the number of visits they received. I had to start with flowers

that had never been visited, so each night I would either put wax-paper bags over flowers on plants in the wild, or I would clip flowers and place them in florist's cut-flower holders inside large styrofoam boxes. This was done for both buds that would enter male phase overnight and male-phase flowers that would enter female phase. Such flowers appeared normal in every way. The next day I would allow bees to visit the flowers. The bagged flowers were unbagged and, in some experiments, watched until they were visited as prescribed by the experimental design. Alternatively, they were cut and put into cut-flower holders. Flowers in holders were presented to bees on the end of a stick (Thomson, 1981). Foraging bees generally move from flower to flower, and do not seem to mind if the flower is in a holder on the end of a stick held by a researcher. Often the duration of the visit was timed with a stop watch, but visit duration has proven to be inconsequential, so it will not be reported on here. Unvisited control flowers were included in all experiments. After a flower had been visited or not depending on treatment, the androecium (if it was in male phase) was placed in a microcentrifuge tube, dried, and preserved in ethyl alcohol; or the stigma (if it was in female phase) was squashed in fuchsin-tinted glycerin jelly between a microscope slide and a cover slip (Beattie, 1971b).

Pollen left in anthers from the 1988 and 1989 seasons was counted with a Coulter[®] electronic particle counter (see Wilson & Thomson, 1991, for details). Pollen from subsequent years was counted using an Elzone[®] counter equipped with a 76 μ aperture. Pollen was diluted into 200 ml of 1% saline solution, sonicated in an ultrasonic water bath to break up clumps of pollen, and all particles larger than 16 μ were counted for three 0.3 ml subsamples. The average of these three counts was multiplied by the dilution factor to estimate the number of pollen grains left in the androecium. The number of pollen grains left in androecia can be thought of as the opposite of the number removed, and in several places I find it useful to present the number removed by subtracting the number left after a visit from the number produced in unvisited flowers.

Pollen deposited on stigmas was counted by eye through a compound microscope equipped with an ocular grid. The entire area of the cover slip was scanned swath by swath. Pollen on stigmas was generally log normally distributed and was transformed by $\log(\text{pollen deposited} + 1)$.

For field seasons after 1989, perianths were also preserved in alcohol and later measured for six dimensions, shown in Figure 1. This was done using a dissecting microscope with the flower floating in alcohol in a petri dish over 1 mm graph paper. The width and height of the mouth of the vestibular sepal, a and b , was thought to represent how tightly the flower must fit around the bee at the point where the androecium or stigma is located. These two dimensions are rather difficult to estimate because they have to be measured on the flower while it is in its 3-dimensional state. For this reason, I also measured the height of the mouth of the vestibule after it was gently flattened, c . The roof and floor length of the vestibule, d and e , were measured to represent how far into the flower a bee must crawl into the vestibule before her tongue can be bent into the spur. The height of a porch petal, f , was the final measurement taken, and was thought to be important because bees stand on it and push down against it when crawling into

TABLE 1. Study sites and years with locality information. Each sample is coded, and codes are used in subsequent tables.

<i>Impatiens pallida</i> samples	
p-1:	Maple Gap 1990 – Albany Co., Rensselaerville, on the Huyck Preserve, along Trout Creek, 0.5 km up from Lake Myosotis.
p-2:	New Salem 1990 – Albany Co., New Salem, 0.5 km nw of the nw corner of the Reservoir.
p-3:	Maple Gap 1991 – same as p-1.
p-4:	Trout Creek 1991 – 0.5 km farther up Trout Creek than p-1.
p-5:	Livingstonville 1991 – Schoharie Co., Livingstonville, along Lake Creek, 2 km from its confluence with Catskill Creek.
p-6:	Reservoir 1992 – Albany Co., New Salem, above e side of the Reservoir, 1 km se of p-2.
p-7:	Freehold 1992 – Greene Co., Freehold, along road 67, next to bridge to East Durham.
<i>Impatiens capensis</i> samples	
c-1:	Weld 1988 – Suffolk Co., Nissequogue, on the Weld Preserve, west edge.
c-2:	Weld 1989 – same as c-1.
c-3:	Weld 1990 – same as c-2.
c-4:	Barn 1990 – on the Huyck Preserve, above ne corner of Lake Myosotis.
c-5:	Lincoln Pond – next to Lincoln Pond, 1 km n of c-4.
c-6:	Strocks' 1990 – Rensselaerville, just w of Huyck Preserve, 1 km w of c-5.
c-7:	Livingstonville 1991 – same as p-5.
c-8:	Woods Road 1991 – less than 0.5 km n of c-6.
c-9:	Rensselaerville 1992 – 0.5 km s of c-4.
c-10:	Albany 1992 – Albany Co., Albany, on SUNY campus.

flowers. Several synthetic variables were computed as well. The displacement between the roof and the floor length, $g = d - e$, seemed likely to account for the degree to which a bee could crawl past the sexual parts without making contact. The value $h = \log(a) + \log(b)$ was used as an index of the vestibule entrance area. The mean of the three flattened vestibule dimensions, $i = (c + d + e) \div 3$, was used as a general summary of vestibule size that can be easily thought about since it is in millimetres. My hypothesis was that the smaller c , f , g , h or i the more tightly the flower fits around the bee and the more pollen ought to be transferred in a visit.

In order to randomize measurement biases, androecia, stigmas and perianths were scored in random order and without knowledge of experimental treatment. For some of the pollen counts, the randomization only included flowers within an experiment; however, pollen counts are extremely repeatable, so there should be no problem with comparing experiments. For the perianth measurements and many of the pollen counts, the randomization included all data sets within a year. The work was done in New York between 1988 and 1992, mostly in Albany County, with one site in adjacent Schoharie County (Livingstonville), one in adjacent Greene County (Freehold), and one in a distant site on Long Island (Weld Preserve). Years and sites are listed in Table 1.

PRELIMINARY STUDIES

Means, correlations, and standard deviations

Table 2 shows the means of the eight floral dimensions for the 15 site-year data sets of 1990–1992. There were some fairly substantial differences among sites. For instance, in the synthetic size variable i , there was a range

TABLE 2. Means of perianth measurements (Figure 1) for fifteen site-year samples of *Impatiens pallida* (p-) and *I. capensis* (c-).

	min(<i>N</i>)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>
p-1	(153)	13.7	9.5	19.1	15.1	19.4	6.7	-4.3	2.107	17.9
p-2	(364)	17.8	10.7	22.7	16.4	21.3	7.4	-4.9	2.278	20.1
p-3	(132)	9.7	12.6	18.1	14.6	18.3	7.0	-3.7	2.081	17.0
p-4	(66)	9.4	13.0	18.2	14.3	17.9	7.1	-3.6	2.082	16.8
p-5	(201)	11.3	16.4	21.6	17.0	20.0	8.5	0.04	2.265	19.5
p-6	(197)	11.2	15.7	21.8	16.0	20.7	7.9	4.7	2.242	19.5
p-7	(187)	10.7	14.2	19.1	16.3	19.2	7.9	-2.9	2.175	18.1
c-3	(179)	13.1	10.2	16.9	18.6	19.0	6.5	-0.4	2.122	18.2
c-4	(208)	12.8	9.2	16.3	20.0	18.9	7.2	1.2	2.068	18.4
c-5	(55)	11.9	9.3	15.5	19.0	18.7	6.3	0.3	2.038	17.8
c-6	(124)	12.3	9.1	15.7	17.8	17.2	6.2	0.6	2.042	16.9
c-7	(120)	9.2	11.0	14.8	17.6	18.3	6.5	0.6	1.999	16.9
c-8	(146)	8.3	11.4	14.7	18.4	17.9	6.4	0.4	1.966	17.0
c-9	(88)	8.5	10.4	14.3	18.0	17.9	6.7	0.09	1.943	16.7
c-10	(62)	10.2	12.2	16.3	19.1	19.8	6.7	-0.7	2.093	18.4

among site means of 3 mm, and in the primary measurements the means ranged across a fifth of their value by as much as 4 mm.

I calculated standard deviations and correlations within sites for both species, as shown in Table 3. This was done by subtracting the mean of each population from each raw number before doing further computations. The standard deviations given along the diagonal of Table 3 show that there was a limited amount of variability within populations. Subjectively, in the field, *Impatiens* flowers seem to vary considerably. Evidently, however, most of the flowers are within a couple of millimetres of the mean for any one

TABLE 3. Within-population correlations and [standard deviations] for the six morphometric dimensions (see Figure 1). The population means were subtracted before all other computations. Numbers from 1298 *Impatiens pallida* flowers are shown above the diagonal; those from 977 *I. capensis* flowers are shown below the diagonal. All correlations are significantly positive.

<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>
[1.34 mm]	0.34	0.62	0.36	0.41	0.31
[1.11]	[1.43]	0.72	0.31	0.53	0.37
0.28	[1.10]	[1.75]	0.41	0.66	0.42
0.64	0.62	[1.24]	[0.86]	0.49	0.36
0.37	0.32	0.45	[0.98]	[1.69]	0.34
0.37	0.27	0.44	0.63	[1.18]	[0.58]
0.30	0.23	0.32	0.35	0.26	[0.54]

population, and this variance is substantially less than that found among populations.

All the correlations among the six primary measurements were significantly positive (Table 3). These correlations are fairly low for morphometric data, which is at least partially because of measurement error that is unavoidable in such soft-bodied specimens. The correlations between the flattened entrance height c and the 3-dimensional-entrance measurements a and b were reassuringly high, but curiously, a and b were not very highly correlated. The two vestibule-depth measurements d and e were also fairly highly correlated, but neither was particularly tightly correlated with c . The low correlations among the floral dimensions suggest that there could be some opportunity for selection to act independently on several aspects of the way the flowers fit around bees' bodies.

Nested levels of variance

Much of my study focuses on the size of flowers, and it is therefore of interest to know how my measurements vary due to estimation error as opposed to real differences among flowers and plants. For each plant species, 10 plants in one population were chosen haphazardly. On each plant, five flowers were bagged in bud. The next day the flowers were unbagged, the androecium was carefully removed into a microcentrifuge tube, and the rest of the flower was preserved in alcohol. Each flower was measured twice in random order among all other flowers, allowing me to estimate the amount of variance in dimensions that was due to error, to differences among flowers on a plant, and to differences among plants in a population. In these same flowers, the amount of pollen produced was estimated using the particle counter.

Table 4 shows nested analyses of variance for pollen production and the six morphometric variables. The counting of pollen in androecia was repeatable with only 2% of the variance being due to subsample. All further reporting of pollen counts is from averages of three such subsamples. Measurement error was substantial for height a and width b of the vestibule entrance, the standard deviation among measurements (root mean square) being on the order of 0.5 mm and the percent variance component between replicate measurements being in the double digits. There was considerably less error in the other four measurements. For the flattened vestibule dimensions c , d and e , the variance component within was always less than 10%. For porch height f , the variance among measurements was somewhat larger when expressed as a percent, but that is because this dimension was less variable among flowers and among plants.

For pollen production and all the morphometric dimensions, both flowers and plants varied significantly. For the flattened vestibule dimensions c , d and e , differences from plant to plant explained much more than half the variance. This is consistent with the possibility that flower size and shape vary genetically within populations, although confirming this would require a breeding experiment.

TABLE 4. Variance components for pollen production and six perianth dimensions (see Figure 1) among plants (top number), among flowers within a plant (second number down), and among replicate measurements of a flower (third number). All given as percentages of the total. [Root mean square given in brackets.]

Pollen production	<i>Impatiens pallida</i>					
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>
41.9%***	47.3****	41.7***	62.8****	71.0****	64.2****	23.8*
56.2****	28.6****	47.7****	34.1****	26.4****	27.8****	60.4****
1.9	24.1	10.7	3.0	2.7	8.0	15.7
[23229 grains]	[0.406 mm]	[0.552 mm]	[0.230 mm]	[0.133 mm]	[0.396 mm]	[0.198 mm]
pollen production	<i>Impatiens capensis</i>					
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>
56.6****	26.2**	61.3****	59.8****	61.7****	80.4****	66.4****
41.1****	58.7****	28.1****	37.3****	30.8****	17.6****	19.3****
2.3	15.1	10.6	2.9	7.5	2.0	14.3
[12101]	[0.331]	[0.420]	[0.210]	[0.265]	[0.172]	[0.181]

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Variance among plants was tested over flowers within a plant, and flowers within a plant over measurements of a flower: these are nested pure model II ANOVAs. For each species, there were 10 plants with 5 flowers in each. The perianths were measured twice with all measurements within a species done in random order. Three pollen subsamples of 0.3 ml from a 200 ml dilution were counted; these counts were not done in random order.

Androecial depletion and stigmatic saturation

I had several reasons to be interested in the number of visits it takes to remove all the pollen that can be removed and to deposit all that can be deposited. First, these are fundamental floral functions. Until rather recently, it was not known how much pollen bees remove in a visit, and it was a surprise to find when the first few measurements were made that a bumblebee removes a fairly high proportion of the grains that can be removed in a single visit (bee results summarized and compared to hummingbirds by Mitchell & Waser, 1992). The following data more or less fit this generality, which is interesting in its own right because it has implications for gender-biased selection (Wilson *et al.*, 1994). It also establishes that most of pollen transfer happens in the first visit, clarifying the interpretation of my other experiments on flowers that were only visited once.

Flowers were covered in the late afternoon with wax paper bags folded so as to not interfere with the flower opening or the androecium being shed. The next day, flowers were unbagged one at a time and allowed to be visited 0, 1, 2, 3, or in the case of *I. capensis* 4 times. These treatments were interspersed in *I. capensis* and were more properly preassigned randomly in *I. pallida*. After a flower received its allotted number of visits, it was picked, and the androecium or the stigma was preserved for later pollen counts.

For both species, a substantial amount of the pollen was removed and deposited in the first visit. In the *Impatiens pallida* study, 27% of the pollen produced was removed after one visit, and there was significant removal after two and three visits such that 50% of the pollen had been removed after three visits (Fig. 2). In the *Impatiens capensis* study, there was not much additional removal after the first visit (Fig. 3). In neither *I. pallida* nor *I. capensis* was there significant deposition of pollen after the first visit, which

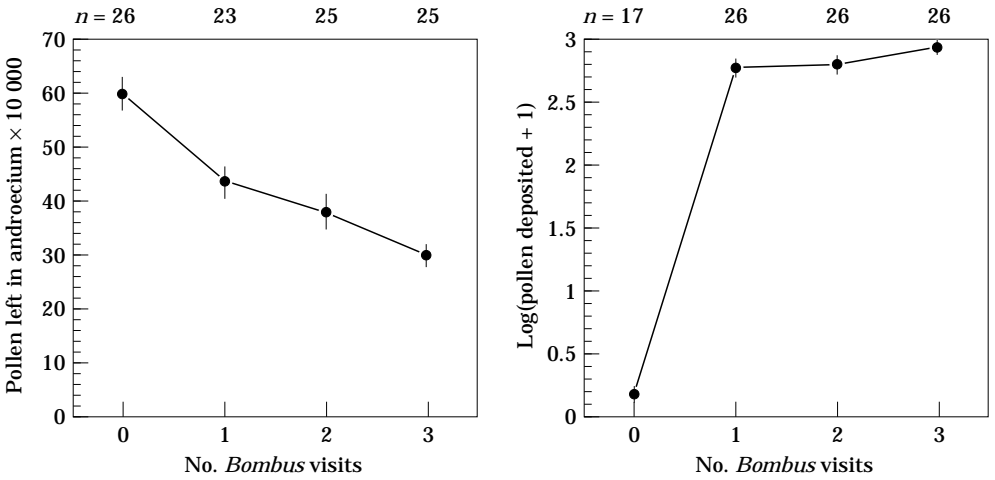


Figure 2. Androecial depletion and stigmatic saturation of *Impatiens pallida* by *Bombus* spp. Points and bars are means ±SE.

generally landed more than 100 grains. Such a first visit after being unbagged is analogous to the first visit of the morning, and my other experiments are on this sort of visit. I recognize that important aspects of the pollination process can occur when there is frequent pollinator visitation during gradual pollen presentation (Harder & Thomson, 1989; Wilson *et al.*, 1994), but here I deal only with the initial visit after an absence of pollinators.

FUNCTIONAL EFFECTS OF ARTIFICIAL CHARACTER VARIATION

Based on the hypothesis of physical fit, it seemed reasonable to think that several nuances of structure in *Impatiens* ought to determine how tightly flowers fit around bees and therefore how much pollen is removed or

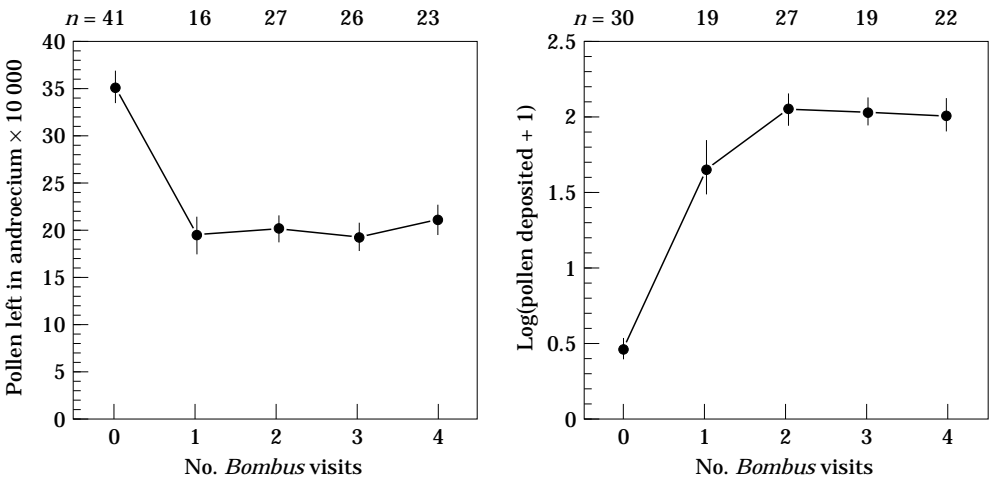


Figure 3. Androecial depletion and stigmatic saturation of *Impatiens capensis* by *Bombus* spp. Points and bars are means ±SE.

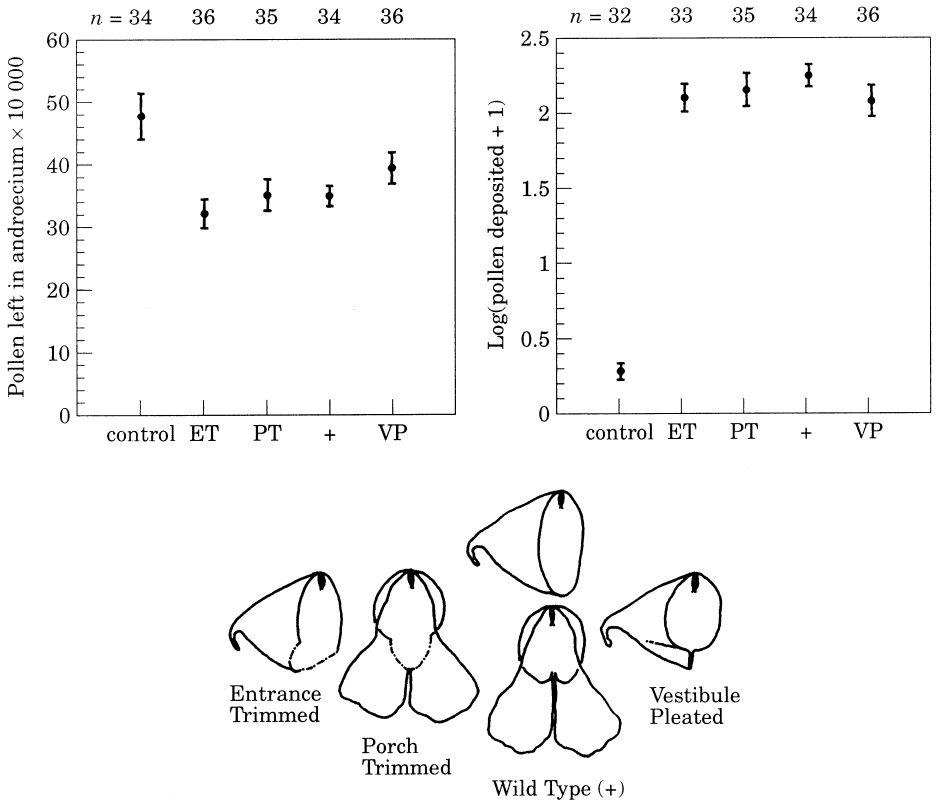


Figure 4. Pollen removal from and deposition on *Impatiens pallida* flowers of various altered morphologies. Points and bars and means \pm SE. The order of treatments reflects predictions based on hypothesized amount of contact. The treatments did not, however, differ significantly from each other. For pollen left, the mean square among the four non-control groups was 3.069×10^{10} (with 3 df), and the root mean square error was 1.333×10^5 (137), yielding $F = 1.73$ and $R^2 = 0.04$. For pollen deposited, the mean square among the four non-control groups was 0.19577 (3), and the root mean square error was 0.57233 (134), yielding $F = 0.60$ and $R^2 = 0.01$.

deposited in a visit. I decided to alter the flowers with scissors and glue to test my predictions. Recently, such use of artificial character variation has been adopted by many behavioural ecologists (Andersson, 1982; Basalo, 1990; Grafen, 1988; Reeve & Sherman, 1993). There is also a long tradition among floral biologists (Clements & Long, 1923; Waser & Price, 1983; Bell, 1985). Most of this work has looked at the effect of altered morphologies on fitness via visitation rates. In contrast, my study is concerned with the amount of pollen transfer between bee and anthers or stigmas given that a visit did occur.

There were five treatments: unvisited flowers, unaltered flowers, flowers with the lower lip of the vestibule trimmed, flowers with a pleat in the floor of the vestibule to reduce girth, and flowers with porch petals trimmed. The trimming was done with a pair of cuticle scissors. The pleats were made by tucking the floor of the vestibule with a tiny drop of Superglue[®]. The various morphologies are shown in Figures 4 and 5. (Note that although porch petals are not shown on some of the illustrations, they were always present—only

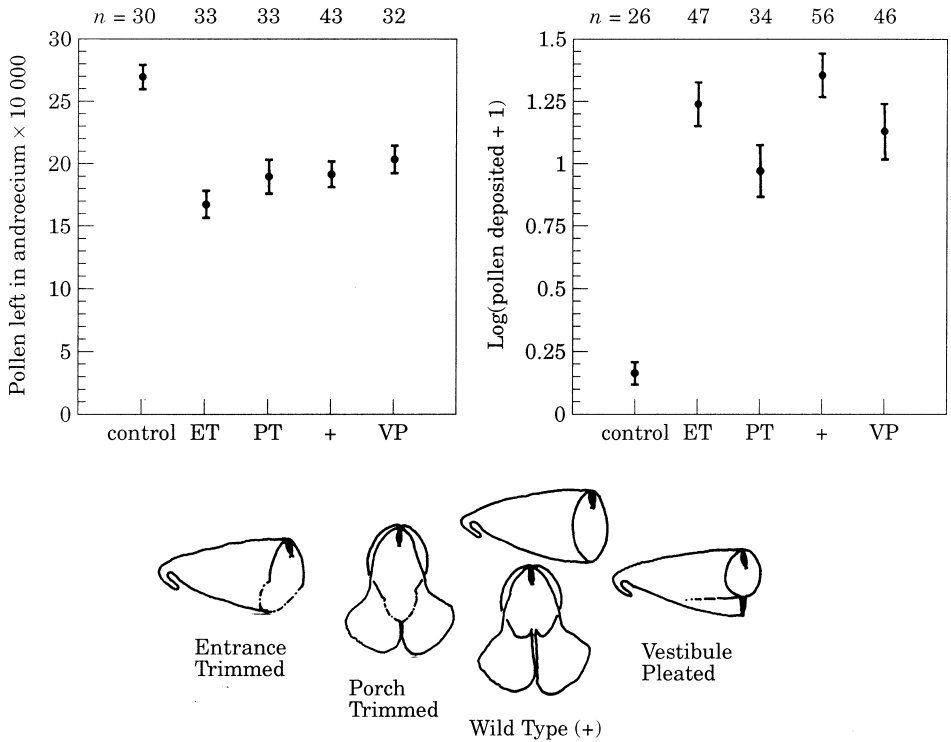


Figure 5. Pollen removal from and pollen deposition on *Impatiens capensis* flowers of various altered and natural morphologies. Points and bars are means \pm SE. The order of treatments reflects predictions based on hypothesized amount of contact. For pollen left, there was no significant difference among treatments; the mean square among the four non-control groups was 7.457×10^9 (with 3 df), and the root mean square error was 6.721×10^4 (137) yielding $F = 1.65$ and $R^2 = 0.04$. For pollen deposited, there was a marginally significant difference among the treatments ($P = 0.051$); the mean square among the four non-control groups was 1.14265 (3), and the root mean square error was 0.65932 (179), yielding $F = 2.63$ and $R^2 = 0.04$; the Tukey multiple comparison revealed that PT was different from +.

in the last treatment were they cut and then only the lip that the bees normally stand on.) I predicted, first, that the order of the treatments would be the same for both pollen removal and pollen deposition. Second, I predicted that the order of the treatments would be the same in the two species. Third, I predicted that both of the trimming treatments would lead to less contact and less pollen removed or deposited, and that the pleating treatment would lead to more contact and more pollen movement. Since the bees stand on the lip of the porch petal and the floor of the vestibule, the trimming of these structures should have meant that the bees did not have to crawl into as confined an opening or press as tightly up against the low-hanging androecia and stigmas; conversely the pleating should have constricted entry more.

Each afternoon, buds and male-phase flowers were cut and placed in cut-flower holders in styrofoam boxes. The next morning, flowers that had matured were randomly assigned treatments and altered if necessary. They were presented for one visit to a naturally foraging *Bombus vagans*. The visits seemed in every way normal, with the bee crawling into the flower and

drinking nectar. After the visit, the flower parts were preserved for eventual scoring.

Figures 4 and 5 show the results. The treatments are ordered from left to right following my predictions. None of those predictions were consistently sustained, not even the prediction that the ranking of the treatments would be the same for pollen left as for pollen deposited. For *Impatiens pallida*, I did not detect any significant differences among treatments for pollen left or for pollen deposited. For *I. capensis*, there were no significant differences among treatments for pollen left, but an effect was detected for pollen deposited, with the only significant multiple comparison being between porch petals trimmed (PT) and normal flowers (+) (see captions of Figures 4 and 5 for ANOVAs.) For pollen left in androecia, the ranking of the treatments is the same in the two studies and the opposite of what I predicted, although when the two data sets were combined in a two-way ANOVA of species by treatment, there was still no significant effect of treatment ($P = 0.215$).

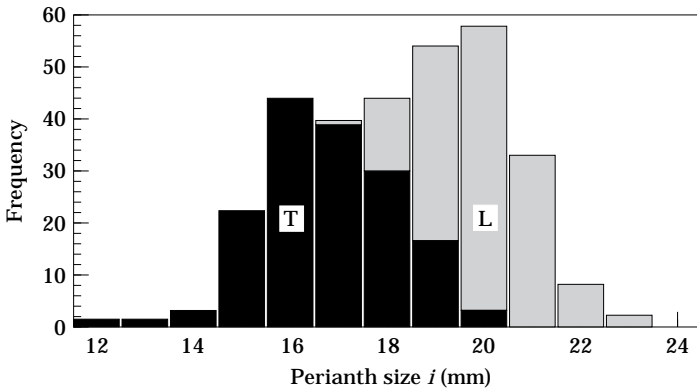
POPULATION AND SPECIES CONTRASTS

Reciprocal transfer experiments

There is a long tradition of reciprocal transplant experiments in the study of local adaptation (Turesson, 1922; Clausen *et al.*, 1940; Antonovics *et al.*, 1971). Such experiments have almost always been done to separate genetic from environmental contributions to vegetative growth and morphology. For instance, Schemske (1984) and Schmitt & Gambel (1990) have found very local adaptation for growth and reproduction in jewelweeds. Since after my first two field seasons I was sure that jewelweeds also vary considerably among sites in flower dimensions and in the amount of pollen moved (see next section), I was interested in finding out whether the differences in pollen moved are the result of different flower sizes or are caused directly by the pollinator regime at a site.

For each jewelweed species, I chose a site with particularly large flowers and a site with particularly small flowers. *Impatiens pallida* was studied in 1991, with the small-flower site being at Trout Creek (p-4) and the large-flowered site at Livingstonville (p-5). *Impatiens capensis* was studied in 1992, with the small-flower site being at Rensselaerville (c-9) and the large-flower site at Albany (c-10). Each afternoon, I would cut flowers from both source sites in bud and in male-phase and put them in cut-flower holders in styrofoam ice chests. The next morning, they were taken to one of the two test locales, each flower that had matured was presented to a *Bombus vagans* for a single visit, and then its parts were preserved. Flowers from the two source sites were alternately presented, so source can be treated as randomized (actually interspersed). Locale, however, was not randomized since it was not feasible to switch back and forth between locales. This is therefore a split-plot design without replication of locale, and it is formally improper to test for locale effects.

For *Impatiens pallida*, Livingstonville flowers were much larger than Trout Creek flowers, as is evident from the histogram of the size variable i shown



		POLLEN REMOVED		POLLEN DEPOSITED	
		Source site		Source site	
		L	T	L	T
Locale site	L	869 ± 27.4 (13) 325 ± 17.8 (27) 544 = 63%	397 ± 35.4 (14) 254 ± 25.8 (27) 143 = 36%	0.70 ± 0.145 (16) 2.08 ± 0.070 (32) 115	0.37 ± 0.108 (16) 2.46 ± 0.051 (32) 286
	T	742 ± 43.2 (14) 466 ± 26.0 (28) 276 = 37%	482 ± 46.7 (13) 318 ± 23.8 (28) 164 = 34%	0.77 ± 0.215 (15) 2.45 ± 0.077 (32) 276	0.52 ± 0.081 (18) 2.72 ± 0.070 (34) 521

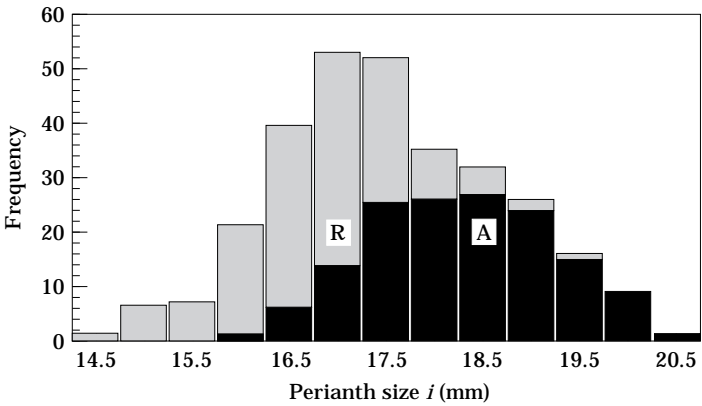
Figure 6. Reciprocal transfer of *Impatiens pallida* flowers between Trout Creek (T, ■) and Livingstonville (L, □). The histogram (above) of dimension *i*, perianth size, shows that the flowers from L were larger than those from T.

In the table on the left, the large numbers are my best estimate of the number of pollen grains removed ($\times 1000$) in a visit by a *Bombus vagans*. This estimate was calculated as the difference between the average number of pollen grains produced in unvisited control flowers [first line of small numbers in each cell on the left] and the average number of pollen grains left in androecia after one visit [second line of small numbers in each cell on the left]. Means are $\times 1000 \pm SE$ (sample sizes).

In the table on the right, the large numbers are my best estimate of the number of pollen grains deposited in a visit by a *Bombus vagans*. This estimate was calculated as the difference between the antilog of the average log (pollen on unvisited stigmas + 1) [first line of small numbers in each cell on the right] and the antilog of the average log (pollen deposited on stigmas of visited flowers + 1) [second line of small numbers in each cell on the right]. Means are in log units $\pm SE$ (sample sizes).

at the top of Figure 6. In the lower half of the figure, average pollen counts are tabulated by source site and by locale site, and are shown in small print. The numbers in large print are my best estimate of the amount of pollen removed and deposited; these values were calculated by subtracting the mean values for the control flowers from the mean values for the visited flowers. Two-way ANOVAs, however, were done directly using data from only the visited flowers: for both removal and deposition, there were highly significant ($P < 0.01$) effects of source site and locale site (not properly randomized) but no significant interaction ($P > 0.05$).

The results for *Impatiens capensis* are given in Figure 7. In this case, flowers from the two populations did not differ quite as much in size. For the ANOVAs, the only significant term ($P < 0.05$) was source site for pollen left in androecia, which is not necessarily due to floral morphology since the flowers from the two sites produced different amounts of pollen to begin



		POLLEN REMOVED		POLLEN DEPOSITED	
		Source site		Source site	
		A	R	A	R
Locale site	A	392 ± 18.5 (15) 250 ± 15.9 (31) 142 = 36%	292 ± 16.2 (16) 176 ± 12.4 (31) 116 = 40%	0.17 ± 0.169 (5) 1.57 ± 0.283 (11) 36	0 (5) 1.38 ± 0.350 (11) 23
	R	407 ± 25.1 (16) 258 ± 16.2 (32) 149 = 37%	340 ± 13.0 (16) 181 ± 9.3 (33) 159 = 47%	0.10 ± 0.050 (9) 1.53 ± 0.133 (30) 33	0 (9) 1.78 ± 0.108 (30) 59

Figure 7. Reciprocal transfer of *Impatiens capensis* flowers between Albany (A, ■) and Rensselaerville (R, □). Arrangement of numbers as in Figure 6.

with. For pollen deposited, sample sizes were low at the Albany site, basically because of a fluctuation in bee abundance.

The significant source effects, where they were evident, suggest that some characteristics of the flowers differed between the sites in affecting how much pollen was left in androecia or deposited on stigmas. This could be flower size, which differed more dramatically between the *Impatiens pallida* sites where there were large source effects, than between the *I. capensis* sites, where there were not. If flower size and perianth morphology were responsible, this would represent local divergence of the mechanical fit between flower and pollinator. Sadly, there are many other features of the flowers that might be responsible for the source effects. The difference in amount of pollen produced surely could have affected how much remained after a visit (Young & Stanton, 1990). Similarly, a difference in stigma size or stickiness could have caused the difference in pollen deposited. It is difficult to say more than that the divergent flowers of distinct sites in *I. pallida* did differ in some functional attribute, and that this did not seem particularly true of the two *I. capensis* sites.

The locale effects, where they were evident, in *Impatiens pallida*, represent differences in how much pollen was moved to and from a flower of a particular type. The locale effects might have been due to the bees somehow behaving differently, or they might have been due to differences in the characteristics of the surrounding jewelweed flowers—for instance, perhaps

those flowers were putting more pollen on the bees' backs. As already stated, locale was not randomized, so experimental artifact is another explanation.

Despite these problems of interpretation, my conclusion from this study is that the differences among sites, which I have often found (see below), are probably due to both the characteristics of the flowers and to the pollen dynamics at a particular site during a particular period of time.

The two Impatiens in sympatry

In the absence of data, one might imagine that *I. pallida* and *I. capensis* have diverged under the influence of different pollinator regimes. Essentially the only features that are different are characteristics of the flowers, namely, perianth colour and shape. It is therefore tempting to think that they are adapted to slightly different pollinators or combinations of pollinators. Another adaptive explanation might be that they first evolved an incompatibility, and then upon coming back together experienced character displacement such that there was floral differentiation to avoid either interference competition or the wastage of energy on seeds that fail to develop (Grant, 1994).

At the Livingstonville site, both species grow microsympatrically, and bees were seen flying between them without any strong discrimination (also see Rust, 1977b). Flowers were cut, put in cut-flower holders, and allowed to change phase inside of styrofoam boxes overnight. The two *Impatiens* species were presented to *Bombus vagans* in alternating order. I also alternated the species that the bee was naturally foraging on when presented with the cut flower.

Surprisingly, there was not much difference between species in the amount of pollen deposited on stigmas (Fig. 8). There was a large difference in the

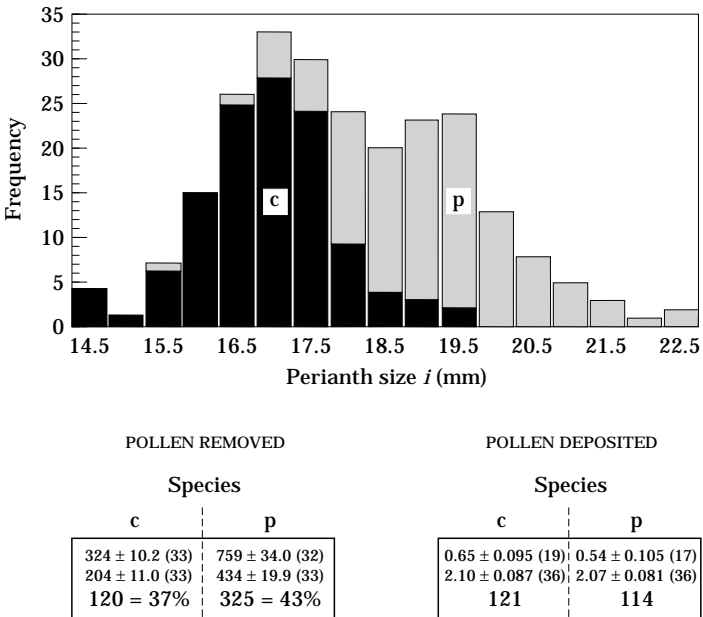


Figure 8. Comparison of *Impatiens pallida* (P, □) and *I. capensis* (C, ■) in sympatry. Arrangement of numbers as in Figure 6.

amount of pollen produced, and again this confounds any exact interpretation of the amount of pollen left. Nevertheless, it would seem that the proportion removed is quite similar for both species, and the number of grains deposited was almost identical. Furthermore, pollen from the two species was placed on and received from the same area of the bee's thorax.

The characteristics of jewelweed flowers are surely much more disparate between the two species than among populations or among individuals within populations. The finding that when the plants that differ the most are intimately juxtaposed there is no resulting disparity in floral function suggests that large differences in amount of pollen moved among sites (see below) are properties of the sites and not of the plants.

WITHIN- AND AMONG-POPULATION ANALYSES

The population thinking demanded by evolutionists has always implied the necessity for empirical work of heroic proportions (Thompson, 1988). Many plant species seem to be characterized by distinctive floral attributes, as *Impatiens pallida* and *I. capensis* so delightfully exemplify, and floral biologists have often relied on a single population to represent a species. Yet what happens in a single population does not inform us about a species as a collection of populations. We would really like to know (1) if the characteristics that distinguish species have diverged functionally (Wilson & Thomson, in press), (2) how selection does or does not vary through space and time (e.g. Weis *et al.*, 1992), and (3) how selection itself changes as populations and species diverge (Armbruster, 1990). *I. pallida* and *I. capensis* have characteristic species-level differences in morphology. On a lesser scale, populations are also well differentiated. Having diverged somewhat, do these species and populations bring, as it were, unique blocks of marble to be sculpted differently by selection?

Table 5 shows the number of pollen grains found in anthers and the number of pollen grains on stigmas for seven samples of *I. pallida* and ten samples of *I. capensis*. The samples are mainly from different sites, but a few are from the same place in different years. Results are given for unvisited (control) flowers and for flowers that were visited once by a bumblebee, usually *Bombus vagans* except where otherwise noted. Percent removed, given in bold print, was calculated as the difference between the means for controls and visited flowers divided by the mean number of pollen grains produced in the controls. Likewise, pollen deposited, also given in bold, was calculated as the difference in the backtransformed means of visited and control flowers.

Although in all cases bees did remove and deposit pollen, there was very significant heterogeneity among samples within species for both removal and deposition. Pollen removal ranged from 14% to 51%, and pollen deposited from 7 grains to 575. The differences between unvisited and visited flowers were subjected to a meta-analysis (Gurevitch & Hedges, 1993). This statistical technique allows one to summarize the results of many separate experiments, which need not even be on the same organisms, and to evaluate whether there is an overall effect of treatment (visited versus unvisited) and whether there are heterogeneities among the different experiments (different sites, different species). Among my samples within species, I found very significant

TABLE 5. Pollen removal and deposition in *Impatiens pallida* (p-) and *I. capensis* (c-). Numbers are means \pm standard errors (with sample sizes. **Bold face** numbers are an estimate of pollen removed and deposited in a single visit by a bumblebee; they were computed as the difference between visited and unvisited (control) flowers.

Code	pollen production and removal			stigma contamination and deposition		
	unvisited fls ¹	visited fls ¹	% removed	unvisited fls ²	visited fls ²	deposited
p-1	961 \pm 70.5 (29)	634 \pm 45.4 (40)	34	0.51 \pm 0.082 (19)	2.27 \pm 0.058 (54)	183
p-2 ³	1326 \pm 32.5 (46)	930 \pm 24.4 (130)	30	0.42 \pm 0.054 (41)	2.57 \pm 0.032 (145)	373
p-3	476 \pm 36.6 (34)	349 \pm 15.7 (34)	27	0.28 \pm 0.056 (32)	2.25 \pm 0.070 (34)	174
p-4	482 \pm 46.7 (13)	318 \pm 23.8 (28)	34	0.52 \pm 0.081 (18)	2.72 \pm 0.070 (34)	516
p-5 ⁴	791 \pm 26.3 (45)	385 \pm 15.2 (60)	51	0.61 \pm 0.088 (33)	2.08 \pm 0.053 (68)	115
p-6	599 \pm 30.1 (26)	436 \pm 31.5 (25)	27	0.15 \pm 0.055 (17)	2.76 \pm 0.079 (26)	575
p-7	759 \pm 25.9 (34)	438 \pm 27.7 (33)	42	0.40 \pm 0.179 (19)	2.36 \pm 0.082 (35)	226
c-1	964 \pm 64.6 (42)	723 \pm 67.1 (25)	25	0.58 \pm 0.098 (37)	2.12 \pm 0.114 (15)	127
c-2	354 \pm 16.8 (41)	196 \pm 19.4 (16)	45	0.47 \pm 0.065 (30)	1.66 \pm 0.179 (19)	43
c-3	747 \pm 33.2 (28)	427 \pm 27.9 (29)	43	0.28 \pm 0.071 (20)	2.40 \pm 0.086 (33)	251
c-4	813 \pm 21.5 (38)	687 \pm 37.7 (31)	15	0.63 \pm 0.090 (34)	1.07 \pm 0.117 (34)	7.4
c-5	623 \pm 48.3 (16)	538 \pm 54.9 (6)	14	0.34 \pm 0.219 (5)	2.05 \pm 0.152 (13)	111
c-6	630 \pm 37.2 (26)	441 \pm 30.7 (34)	30	0.49 \pm 0.128 (20)	2.03 \pm 0.106 (31)	103
c-7	324 \pm 10.2 (33)	204 \pm 11.0 (33)	37	0.65 \pm 0.095 (19)	2.10 \pm 0.087 (36)	123
c-8	266 \pm 9.6 (36)	178 \pm 9.6 (37)	33	0.24 \pm 0.060 (31)	1.43 \pm 0.087 (49)	25
c-9	340 \pm 13.0 (16)	181 \pm 9.3 (33)	47	0 (9)	1.78 \pm 0.108 (30)	61
c-10	392 \pm 18.5 (15)	250 \pm 15.9 (31)	36	0.17 \pm 0.169 (5)	1.57 \pm 0.283 (11)	36

¹ Pollen left in androecia \times 1000.

² $\log(\text{pollen on stigma}+1)$.

³ Flowers visited by *Bombus vagans*, *B. impatiens*, and *B. fervidus* pooled after testing for heterogeneity.

⁴ Flowers from local part of reciprocal transfer experiment and from between species comparison pooled.

heterogeneity (Table 6). For pollen removal, the species did not differ beyond that of the populations, but for pollen deposition they did, with *I. pallida* often having more pollen deposited than *I. capensis* (see *Q* values in Table 6). A further finding was that, among the 17 samples, there was absolutely no detectable correlation between pollen removal and pollen deposition ($r = -0.06$).

For all but the 1988 and 1989 samples, I had measurements on the flowers for which I had pollen counts. Multiple regressions were done for both pollen remaining and the log of pollen deposited onto three floral dimensions: the height of the vestibule entrance when flattened *c*, the height of the porch petals *f*, and the displacement of the roof from the floor of the vestibule *g* (also see Wilson & Thomson, in press). These three measurements are the ones that I had predicted were most likely to determine how tightly the flower fits around the bee and therefore how much pollen ought to get moved. Such multiple regressions have become a popular method for measuring the strength of selection when there are several potential targets of selection, and the regression coefficients are evocatively termed 'selection gradients' (Lande & Arnold, 1983; but see caveats by Mitchell-Olds & Shaw, 1987). The regressions, shown in Table 7, were done for both unvisited control flowers (above) and for visited experimental flowers (below). The *R*² values are consistently low, and the selection gradients were rarely significantly different from zero. That this was true of controls shows that pollen production and stigmatic contamination were not related to flower dimensions as they

TABLE 6. Meta-analysis of pollen removal and deposition (original statistics in Table 5). In all cases bees did remove or deposit pollen, but there was significant heterogeneity in the amount of pollen moved.

code <i>j</i>	pollen removed				log(pollen deposited + 1)				<i>wd</i> ²				
	<i>s_j</i>	<i>J_j</i>	<i>d_j</i>	<i>w_j</i>	<i>wd</i>	<i>wd</i> ²	<i>s_j</i>	<i>J_j</i>		<i>d_j</i>	<i>w_j</i>	<i>wd</i>	<i>wd</i> ²
p-1	328.98	0.99	-0.98	15.042	-14.8	14.53	0.4099	0.99	4.249	5.134	21.81	92.67	
p-2	264.47	1	-1.49	27.975	-41.7	62.18	0.3771	1	5.678	8.4771	48.14	273.3	
p-3	164.2	0.99	-0.76	15.842	-12.1	9.262	0.3668	0.99	5.308	3.648	19.36	102.8	
p-4	140.37	0.98	-1.15	7.7733	-8.91	10.2	0.3874	0.98	5.593	2.5926	14.5	81.09	
p-5	145.73	0.99	-2.77	13.278	-36.7	101.6	0.4603	0.99	3.169	10.556	33.45	106	
p-6	155.46	0.98	-1.03	11.247	-11.6	11.99	0.345	0.98	7.426	1.3539	10.05	74.67	
p-7	155.06	0.99	-2.05	10.994	-22.5	46.03	0.6038	0.99	3.199	5.6833	18.18	58.16	
				106.0	-141.5	255.8					37.445	165.5	788.7
c-1	390.02	0.99	-0.61	15.017	-9.17	5.602	0.5572	0.98	2.722	6.0623	16.5	44.93	
c-2	100.29	0.99	-1.55	9.2533	-14.4	22.34	0.558	0.98	2.098	7.6396	16.03	33.64	
c-3	163.23	0.99	-1.93	9.7093	-18.8	36.3	0.3231	0.96	6.289	0.2467	1.552	9.757	
c-4	171.55	0.99	-0.73	16.027	-11.6	8.453	0.6086	0.99	0.715	15.98	11.42	8.162	
c-5	180.32	0.96	-0.45	4.2764	-1.94	0.879	0.5341	0.95	3.049	1.8684	5.697	17.37	
c-6	183.69	0.99	-1.02	13.077	-13.3	13.49	0.5834	0.98	2.599	6.7343	17.5	45.5	
c-7	60.936	0.99	-1.95	11.198	-21.8	42.41	0.488	0.99	2.929	6.3134	18.49	54.16	
c-8	58.004	0.99	-1.50	14.237	-21.4	32.08	0.5207	0.99	2.263	11.809	26.73	60.49	
c-9	52.974	0.98	-2.95	5.5004	-16.2	47.98	0.5237	0.98	3.33	3.4896	11.62	38.68	
c-10	83.528	0.98	-1.67	7.7357	-12.9	21.6	0.8186	0.95	1.617	2.6837	4.34	7.017	
				106.03	-142	231.1					62.827	129.9	319.7
				<i>d_i</i>	<i>s_i²</i>	<i>Q_w</i>					<i>d_i</i>	<i>s_i²</i>	<i>Q_w</i>
<i>I. pallida</i>				-1.452	0.01	40.34	<i>I. pallida</i>				4.4199	0.027	57.24
<i>I. capensis</i>				-1.335	0.009	42.25	<i>I. capensis</i>				2.9459	0.016	51.19
				16 <i>df</i>	<i>Q_{total}</i> = 83.31****					16 <i>df</i>	<i>Q_{total}</i> = 238.3****		
				15 <i>df</i>	<i>Q_{within}</i> = 82.59****					15 <i>df</i>	<i>Q_{within}</i> = 108.4****		
				1 <i>df</i>	<i>Q_{species}</i> = 0.718 ns					1 <i>df</i>	<i>Q_{species}</i> = 129.9****		

ns $P > 0.01$; **** $P < 0.001$. Statistics follow Gurevitch & Hedges (1993); *d* values are effect sizes; *Q* values quantify heterogeneity and can be compared to χ^2 .

TABLE 7. Selection gradients (i.e. standardized multiple regressions) within populations that search for the target of selection among three floral dimensions (*c*, *f*, and *g*). Primary statistics given in Tables 2, 3 and 4.

Pop.		Pollen in androecium		Log(pollen deposited + 1)	
		Selection Gradient	R^2	Selection Gradient	R^2
p-1	control	$0.71c^* + 0.25f + 0.40g$	0.25†	$0.28c + 0.01f + 0.47g$	0.15
	visited	$0.17c + 0.02f - 0.14g$	0.09	$-0.16c - .16f - 0.22g$	0.07
p-2	control	$0.17c - 0.06f - 0.00g$	0.02	$0.01c - 0.01f + 0.05g$	0.00
	visited	$0.17c† + 0.18f^* + 0.11g$	0.08*	$0.04c + 0.03f + 0.05g$	0.00
p-3	control	$0.38c + 0.13f + 0.18g$	0.14	$0.26c - 0.17f + 0.28g$	0.09
	visited	$0.13c - 0.11f - 0.10g$	0.03	$-0.26c + 0.17f - 0.13g$	0.06
p-4	control	$0.36c + 0.30f + 0.09g$	0.19	$-0.30c + 0.72f† - 0.09g$	0.49
	visited	$0.54c† - 0.20f - 0.24g$	0.37*	$0.46c - 0.59f + 0.33g$	0.21
p-5	control	$0.32c† - 0.00f - 0.12g$	0.14	$0.16c + 0.13f - 0.15g$	0.11
	visited	$0.39c^{**} - 0.23f† + 0.42g^{**}$	0.28**	$-0.26c† - 0.14f - 0.13g$	0.12*
p-6	control	$0.35c - 0.09f - 0.04g$	0.08	$-0.06c - 0.27f + 0.05g$	0.08
	visited	$0.68c^* - 0.13f + 0.06g$	0.44**	$0.08c - 0.03f - 0.29g$	0.11
p-7	control	$0.87c^{**} - 0.20f + 0.15g$	0.48**	$-0.21c - 0.52f^* + 0.04g$	0.43*
	visited	$0.41c† + 0.21f + 0.32g†$	0.25*	$-0.18c + 27f + 0.18g$	0.08
c-3	control	$0.14c + 0.00f - 0.26g$	0.11	$-0.15c - 0.02f + 0.06g$	0.02
	visited	$0.10c + 0.06f - 0.10g$	0.03	$0.24c - 0.17f - 0.39g^*$	0.26*
c-4	control	$0.10c + 0.18f - 0.05g$	0.04	$-0.20c - 0.07f + 0.22g$	0.09
	visited	$-0.17c + 0.20f + 0.17g$	0.07	$0.25c + 0.02f - 0.01g$	0.06
c-5	control	$0.69c^{**} - 0.04f - 0.17g$	0.51*		
	visited	$-0.10c + 0.79f + 0.30g$	0.55	$0.06c + 0.19f - 0.30g$	0.12
c-6	control	$0.26c + 0.30f + 0.09g$	0.22	$-0.04c + 0.07f + 0.20g$	0.06
	visited	$-0.17c + 0.71f^{**} - 0.28g^*$	0.46**	$0.34c - 0.42f† + 0.13g$	0.12
c-7	control	$-0.02c + 0.09f - 0.26g$	0.09	$-0.21c + 0.25f - 0.15g$	0.14
	visited	$0.38c^* + 0.04f + 0.33g†$	0.30*	$-0.05c - 0.26f + 0.27g$	0.17
c-8	control	$0.22c - 0.31f - 0.35g$	0.23†	$0.18c + 0.22f - 0.01g$	0.11
	visited	$0.09c + 0.00f + 0.10g$	0.02	$-0.17c + 0.13f + 0.18g$	0.08
c-9	control	$0.33c + 0.29f + 0.35g$	0.43†		
	visited	$0.36c† - 0.24f - 0.27g$	0.21†	$-0.39c† + 0.04f - 0.21g$	0.16
c-10	control	$-0.36c + 0.21f - 0.16g$	0.16		
	visited	$0.23c + 0.05f + 0.32g†$	0.17	$0.18c - 0.07f - 0.13g$	0.03

† $P < 0.1$; * $P < 0.01$; all others $P > 0.1$. R^2 is the variance in pollen moved explained by the model.

naturally vary within samples. That it is was true for visited flowers indicates that the floral dimensions do not determine to any great extent how much pollen is moved by bees. Some of the regressions were statistically significant for pollen in androecia and a very few for pollen deposited; these suggest that there might sometimes be a weak relationship. The variables that multiple regression identified as being targets of phenotypic selection in those several populations where significant results were found are not consistently the same variables, and even the sign of the regression coefficient was unstable. This suggests that there might have been heterogeneity in the strength or direction of selection among populations, but given the weakness of the relationships, evaluating heterogeneity among multiple regressions is not feasible.

Because of this, I decided to do univariate regressions of pollen in androecium and the log of pollen deposited on the synthetic size variables. The 30 regressions (*b*'s as slopes of regression lines, not as widths of the vestibule) of pollen removed and deposited on *h* for the 15 populations are given in Table 8, where they are compared among populations and between

TABLE 8. A comparison of selection gradients among populations and between species of *Impatiens*. In the body of the table, each line represents a population. At the bottom, overall analyses are presented with the ANOVAs testing for heterogeneity in slopes, not in means.

code	Regressions of pollen in androecium on <i>h</i>							Regressions of log(pollen deposited + 1) on <i>h</i>						
	<i>n</i>	<i>b</i>	Σy^2	Σx^2	Σxy	SS _{expl.}	SS _{unexpl.}	<i>n</i>	<i>b</i>	Σy^2	Σx^2	Σxy	SS _{expl.}	SS _{unexpl.}
p-1	40	689	32.088	0.37783	260435	1.7952	30.2925	54	0.064	9.717	0.41568	0.0265	0.0017	9.7149
p-2	129	1647	99.587	0.42058	692632	11.406	88.1873	145	0.733	22.021	0.53139	0.3894	0.2854	21.7351
p-3	33	177	2.2556	0.25304	44894	0.0797	2.1760	34	1.145	6.037	0.229597	0.2629	0.3010	5.73556
p-4	39	979	9.717	0.28789	281728	2.7659	7.0148	27	0.513	33.320	0.205565	0.1055	0.0542	33.2655
p-5	102	904	62.303	0.44090	398585	3.6033	58.7001	99	0.261	67.821	0.42267	0.1104	0.0288	67.7913
p-6	25	1208	5.9603	0.07234	87405	1.0561	4.9043	26	2.256	4.071	0.04941	0.1115	0.2516	3.8196
p-7	33	1400	8.0735	0.16355	229047	3.2077	4.8659	35	-0.421	7.916	0.29131	-0.1226	0.0516	7.8641
	401		2.01614①		1994728②	23.905	196.141	420			2.14565	0.88375	0.9743	149.926
c-3	29	210	6.3068	0.09523	19948	0.41784	6.2650	33	0.552	7.854	0.13886	0.07660	0.0423	7.8120
c-4	30	376	13.2113	0.15679	58940	0.22157	12.9898	34	-0.1998	15.381	0.08733	-0.01745	0.0035	15.3775
c-5	6	832	0.90257	0.05271	43849	0.36481	0.5378	13	1.391	3.591	0.04970	0.06913	0.0962	3.4946
c-6	34	695	10.5447	0.17904	124388	0.86418	9.6805	31	0.808	10.442	0.24320	0.19646	0.1587	10.2834
c-7	32	299	1.1390	0.25015	74820	0.22379	0.9153	36	0.166	9.463	0.13942	0.02315	0.0038	9.4588
c-8	35	167	1.2283	0.18791	31399	0.52469	1.1758	47	-0.229	16.623	0.282286	-0.06453	0.0148	16.6084
c-9	33	196	0.9061	0.12076	23619	0.46196	0.8599	30	-2.824	10.231	0.117929	-0.3330	0.9405	9.2903
c-10	31	557	2.3435	0.13782	76714	0.42699	1.9165	11	-1.634	8.784	0.02324	-0.0379	0.0621	8.7215
	230		1.18042③		453680④	2.24179	34.3405	235			1.081988	-0.08765	1.3218	81.0465
631			3.19657⑤		2448408⑥	26.147⑦	223.215	655			3.14031	0.81354	2.29263	215.595
			SS _{1b} = ⑥ ² ÷ ⑤ = 18.754⑧								SS _{1b}	0.19636		
			SS _{pop.} = ② ² ÷ ① = 19.735								SS _{pop.}	0.36400		
			+SS _{exp.} = ⑦ ² ÷ ③ = 1.744								+SS _{exp.}	0.00710		
			= SS _{2b's} = 21.479⑨								= SS _{2b's}	0.37110		
			df	SS	F						df	SS	F	
		species = ⑨-⑧	1	2.726	7.590*						1	0.1747	1.180ns	
		pop's in spp. = ⑦-⑥	13	4.668	0.967ns						13	1.9250	0.401ns	
		error within pop's	601	223.2							625	231.0		

For pollen in androecium, all sums of squares $\times 10^{11}$ and slopes $\times 10^3$. *0.05 > P > 0.01; ns not significant. Code is species and sample as in Table 1; n is sample size; b is the slope of pollen movement on floral morphology; Σy^2 is the corrected sum of squares of pollen in androecium or log(pollen deposited+1); Σx^2 is the corrected sum of squares of h ; Σxy is the corrected sum of cross products; SS_{expl.} is $(\Sigma xy)^2 / \Sigma x^2$; SS_{unexpl.} is $\Sigma y^2 - \text{SS}_{\text{expl.}}$.

species. These are comparisons of (unstandardized) selection gradients. If they had been strong and heterogeneous, studying the causes and consequences of the heterogeneity would be the beginning of a research programme (Wilson & Thomson, in press; Wade & Kalisz, 1990). The species were significantly different in their relationships between pollen in androecium and h ; this heterogeneity is interesting but difficult to interpret since we know that the species differ rather radically in how much pollen they typically produce. For pollen deposition, there was no general difference in slopes between the species. For both removal and deposition, populations within species did not vary significantly. A similar analysis (not shown) using the general size variable i yielded no significant differences among the slopes of the 15 populations or between the species (for slopes of pollen not removed on i , the populations $F_{13,608} = 0.553$ and the species $F_{1,13} = 2.054$; for slopes of $\log(\text{pollen deposited} + 1)$ on i , the populations $F_{13,626} = 1.124$ and the species $F_{1,13} = 0.176$). In reporting this lack of a relationship between pollen moved and floral morphology, it is worth noting again that the standard deviations within populations are not particularly large.

In a final attempt to relate pollen movement to floral dimensions, I regressed percent pollen removed and pollen deposited on h and on i among the 15 populations holding species constant. For removal, $b_{rh} = 3.2 \pm 41.20$ ($P = 0.94$), and $b_{ri} = -0.82 \pm 2.95$ ($P = 0.79$), respectively. For deposition, $b_{dh} = 323 \pm 517.7$ ($P = 0.54$) and $b_{di} = 10.8 \pm 37.69$ ($P = 0.78$), respectively.

DISCUSSION

The amount of pollen moved by a bumblebee in a visit to a jewelweed flower is characterized by non-self-determination, unpredictability and contextuality. I began this project thinking that flowers are subject to constant selection to fit their pollinators, and that in jewelweeds the mechanisms of selection on flowers would be based on simple engineering considerations. I was primarily interested in how, after two species have diverged, their differences in morphology might change the engineering parameters and hence the way selection operates. There is a hint of evidence for this in Table 8. However, the stronger message from my data is that mechanical fit has little influence over the amount of pollen removed or deposited. By altering the flowers with scissors and glue, in only one case did I significantly change the amount of pollen moved in a visit (trimming the porch petals reduced pollen deposition), and the direction of many of the other (non-significant) changes was contrary to my expectations. By reciprocal transfer experiments, I found that locale and some (unidentified) features of the flowers both sometimes influence the amount of pollen moved, but even when the flowers were as different as possible—i.e. belonging to the two different species—they had very similar sympatric pollen transfer characteristics. By studying regressions of pollen removal and deposition on floral dimensions, it became evident that floral morphology as it naturally varies within and among populations does little toward determining how much pollen is removed or deposited, and where I did find an effect, again it was not consistent with prior expectations. A single negative result can be dismissed as meaningless, but recurring negatives should be viewed as an opportunity to change one's views. Pollen

transfer in *I. pallida* and *I. capensis* is not governed very much by floral morphology.

It is not clear why my intuition about how mechanical fit should affect pollination success failed. It was a functional interpretation much like that of generations of pollination biologists since Sprengel (1793) and Müller (1883). Plants with more open flowers, one would imagine, must rely even less on the details of morphology to assure that pollen is transferred onto and off of animals. In the case of jewelweeds, it seems possible that the shape of the flower as a funnel with a recurved nectar spur is designed in such a way that all bees are forced past the androecium or stigma regardless of whether the flower is large or small, whether the porch petals are the right length and have their lip perfectly intact, or whether the floor of the vestibule is of a particular size. It may be that bees who are trying to insert their proboscis as far as possible into the spur naturally come to push their head and thorax forward and up against the roof of the vestibule by expanding their legs. This is a possible explanation for why one size of *Bombus* fits all sizes of *Impatiens* (this paper), and vice versa (Wilson & Thomson, in press). If this is true, or if the details of morphology are simply irrelevant to pollen transfer, it leaves open the question of what type of flower one would expect morphology to be stringently selected on to assure pollination success—possibly flowers with stiff columns such as orchids (Dressler, 1968) and *Stylidium* (Armbruster *et al.*, 1994).

Partly, this is a matter of scale (Wilson & Thomson, in press). The morphological alterations I made were relatively minor. Within populations, the flowers varied to a small though noticeable degree. Among populations and between species, there were larger differences in flower size and shape, but even here they are not nearly so extreme as, say, the difference between one of these bee-pollinated jewelweeds and a long-spurred lepidopteran-pollinated *impatiens* (Kato *et al.*, 1991). Quite conceivably, if one went to grander scales of floral differentiation, one would find tighter relationships between pollen movement and morphological design. For instance, Armbruster (1988) has studied how the distance from the resin gland to the anthers and stigmas affects the probability that a bee will contact the anthers and stigmas in many populations of several species of *Dalechampia*. For lack of variation within populations, he did not attempt regressions at that level, but among populations he found modestly strong correlations between floral dimensions and pollinator effectiveness, and among species there were very tight correlations. It is possible that I failed to find firm relationships between morphology and pollen movement because such variance in characters affecting fitness might have been eliminated by a history of stabilizing selection. Thus, Fenster (1991) has used lack of variation in the corolla length of hummingbird pollinated flowers as evidence for past selection. In my study, the height of the porch petals f is remarkably constant among populations and between species as compared with the other variables, and this lack of variance could conceivably be the ghost of selection past (Connell, 1980). Of course, my goal was not to study the history of selection but to study selection in the here and now, which for mechanical fit seemed weak over the range of variation that I studied within populations, among sites, between species, and through artificial alterations of morphology.

My results join a growing list of studies on floral morphology and the amount of pollen moved in a visit. In a flight-cage study by Galen & Stanton (1989), the proportion of pollen removed from *Polemonium* flowers in a bumblebee visit did not depend significantly on corolla flare, corolla length, or style length, nor was the amount of pollen deposited related to these variables, though it was negatively correlated with the difference between style length and corolla length ($R^2 = 0.18$). In *Ipomoea trichocarpa*, Murcia (1990) found that the amount of pollen removed in a bumblebee visit depended on length of longest stamen ($R^2 = 0.43$) but not on corolla length, corolla width, or stigma-anther separation, and she found that pollen deposited depended on stigma-anther separation and corolla width ($R^2 = 0.22$) but not on the length of the stamens or corolla. In *Ipomopsis aggregata*, the number of dye particles (as analogs of pollen grains) dispersed to various stigmas from one visit at a donor flower has been found to depend on corolla width ($R^2 = 0.20$ for dye and 0.44 for pollen itself) but not on corolla length or stigma exertion (Campbell, 1989; Campbell *et al.*, 1991), and the amount of dye received in a visit depended on stigma exertion ($R^2 = 0.05$) but not on corolla width or length (Campbell, 1991). All these studies (which were done mostly in flight cages) along with mine (done with freely foraging bees) suggest that there may sometimes be selection on the way flowers fit around pollinators, that stamen and style lengths may consistently influence the amount of pollen moved (also see Wilson and Thomson, in press), but that often other morphometric variables have little or no influence over how much pollen is removed and deposited.

A number of additional studies have attempted to measure selection on floral characters with more ultimate components of fitness, such as seed set. For female function, this means that selection could be acting through characters affecting success at being visited or at receiving large amounts of high quality pollen, and that either way those effects would be diluted by any weakness in the relationship between pollen deposition and seed set. In *Polemonium*, corolla length and diameter were significant but weak predictors of seed production ($R^2 = 0.17$ and 0.11, respectively; Galen, 1989). In the hummingbird-pollinated *Lobelia cardinalis*, there was a weak dependency of seed set on the distance between the nectary and the stigma ($R^2 = 0.06$), and such a dependency was not found in the bee-pollinated *Lobelia siphilitica* (Johnston, 1991). In *Viola cazortensis*, which is pollinated by hawkmoths, selection on corolla size and shape for seed production was weak but detectable, whereas the only character for which selection was not detectable was spur length, despite the fact that it was more variable (Herrera, 1993): the size and shape of the corolla is presumably selected on through the choosiness of pollinators in deciding whether or not they will visit flowers, whereas spur length would influence fitness through enforcing an effective mechanical fit with the pollinator's proboscis.

Many other studies have been done on how pollinator behaviour responds to small differences in floral traits. The work of classical ethologists using artificial flowers leaves one with the impression that pollinators can be very fastidious about certain characters in judging which flowers they will visit (von Frisch, 1967; Manning, 1956; Gould, 1986). Studies with real flowers and visitation success have supported this belief. Clements & Long (1923)

presented copious data from experiments in which they artificially altered flowers with scissors. In some cases, often when nectaries were made more visible, visitation increased, but in most cases, when the appearance of the flowers was altered, visitation decreased: trimmed *Rubus* flowers received 28% as many visits as normal flowers, trimmed *Rosa* flowers received 31%, and variously trimmed *Monarda* flowers received only 19%. More recent studies documenting relationships between natural character variation and visitation success have revealed a range of effect sizes. Stuessy *et al.* (1986) found that plucking off the sterile ray florets in patches of *Helianthus grosseserratus* led to visitation rates that were 61% lower than in patches with normal inflorescences. In studies of *Drosera tracyi*, trimming down petal size similarly had a large effect on visitation rate and total (not per visit) pollen removal and deposition (Wilson, in press). Waser & Price (1983) found that in experimental arrays albino *Delphinium nelsoni* were visited at rate of 83% as often as normal blue flowers. Campbell *et al.*'s (1991) data on corolla width in *Ipomopsis* showed only a very weak though sometimes significant relationship with number of visits garnered in the field, and the effect was probably less than the effect of corolla width on pollen export in the flight cage. In *Polemonium*, Galen & Newport (1987) found that the probability of being visited was very significantly and positively associated with flower size (R^2 not available) while there was a weaker negative correlation due to mechanical fit ($R^2 = 0.14$); Galen and Newport were able to calculate the overall expected effect on seed production and to conclude that the effect "due to bumblebee preference for large-flowered individuals should outweigh the associated decrease in pollination efficiency."

If results such as mine are representative of the extent to which perianth morphology affects the mechanical transfer of pollen from anthers to animals and from animals to stigmas, and *if* it is true that animal behaviours such as pollinator visitation and constancy are very tightly influenced by floral characters, then a possible explanation for the reason why flowers are like animals is because they, unlike most organs in plants, experience selection on signalling characters that are interpreted by animals (West-Eberhard, 1983; Endler, 1992). Admittedly, even tiny amounts of selection are sufficient to result in dramatic evolution or stringent phenotypic stabilization in sizeable populations (Simpson, 1953; Lande, 1976; Falconer, 1989). It nevertheless seems possible that when there is a tradeoff between selection for mechanical fit and selection for visitation success, that the latter will be much more deterministic and therefore the principal governor of evolution. Moreover, stringent selection through one path (visitation) will weigh more heavily than weak selection through another path (mechanical fit) in the gigantic equation of all the genetical, energetic, and selective factors that determine phenotypic representation in a population. The question, then, remains, *Why it is that flowers are so orderly when pollination seems to be such a messy process?*

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REFERENCES

- Andersson M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature* **299**: 818–820.
- Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* **7**: 1–85.
- Armbruster WS. 1988. Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. *Ecology* **69**: 1746–1761.
- Armbruster WS. 1990. Estimating and testing the shapes of adaptive surfaces: the morphology and pollination of *Dalechampia* blossoms. *American Naturalist* **135**: 14–31.
- Armbruster WS, Edwards ME, Debrevec EM. 1994. Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology* **75**: 315–329.
- Arnold SJ. 1983. Morphology, performance and fitness. *American Zoologist* **23**: 347–361.
- Basolo AL. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**: 808–810.
- Beattie AJ. 1971a. Pollination mechanisms in *Viola*. *New Phytologist* **70**: 343–360.
- Beattie AJ. 1971b. A technique for the study of insect-borne pollen. *Pan Pacific Entomologist* **47**: 82.
- Bell G. 1985. On the function of flowers. *Proceedings of the Royal Society of London, Series B* **224**: 223–265.
- Bell G, Lefebvre L, Giraldeau L-A, Weary D. 1984. Partial preference of insects for the male flowers of an annual herb. *Oecologia* **64**: 287–294.
- Bertin RL. 1982. The ruby-throated hummingbird and its major food plants: ranges, flowering phenology, and migration. *Canadian Journal of Zoology* **60**: 210–219.
- Brantjes NBM. 1982. Pollen placement and reproductive isolation between two Brazilian *Polygala* species (Polygalaceae). *Plant Systematics and Evolution* **141**: 41–52.
- Brassard JT, Schoen DJ. 1990. Analysis of phenotypic selection among locations in *Impatiens pallida* and *Impatiens capensis*. *Canadian Journal of Botany* **68**: 1098–1105.
- Campbell DR. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. *Evolution* **43**: 318–334.
- Campbell DR. 1991. Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *American Naturalist* **137**: 713–737.
- Campbell DR, Waser NM, Price MV, Lynch EA, Mitchell RJ. 1991. Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution* **45**: 1458–1467.
- Clausen J, Keck DD, Hiesey WM. 1940. *Experimental studies on the nature of species. I. Effect of varied environments on western North American plants*. Carnegie Institution of Washington.
- Clements FE, Long FL. 1923. *Experimental Pollination: an outline of the ecology of flowers and insects*. Carnegie Institution of Washington.
- Connell JH. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos* **35**: 131–138.
- Crepet WL. 1984. Advanced (constant) insect pollination mechanisms: patterns of evolution and implications vis-a-vis angiosperm diversity. *Annals of the Missouri Botanical Garden* **71**: 607–630.
- Davis PH, Heywood VH. 1963. *Principles of angiosperm taxonomy*. New York: Van Nostrand.
- Dobzhansky T. 1937. *Genetics and the origin of species*. New York: Columbia University Press.
- Dressler RL. 1968. Pollination by euglossine bees. *Evolution* **43**: 1137–1156.
- Endler JA. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist* **139**: S125–S153.
- Falconer DS. 1989. *Introduction to quantitative genetics*, 3rd ed. London: Longman.
- Fenster CB. 1991. Selection on floral morphology by hummingbirds. *Biotropica* **23**: 98–101.
- Futuyma DJ. 1986. *Evolutionary biology*. Sunderland, MA: Sinauer.
- Galen C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* **43**, 882–890.
- Galen C, Newport MEA. 1987. Bumblebee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. *Oecologia* **74**: 20–23.
- Galen C, Stanton ML. 1989. Bumblebee pollination and floral morphology: factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). *American Journal of Botany* **76**: 419–426.

- Grafen A.** 1988. On the uses of data on lifetime reproductive success. In: Clutton-Brock TH, ed. *Reproductive success*. Chicago: University of Chicago Press, 454–471.
- Gould JL.** 1986. Pattern learning by honey bees. *Animal Behavior* **34**: 990–997.
- Grant V.** 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**: 82–97.
- Grant V.** 1994. Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Science USA* **91**: 3–10.
- Gurevitch J, Hedges LV.** 1993. Meta-analysis: combining the results of independent experiments. In Scheiner SM, Gurevitch J, eds. *Design and analysis of ecological experiments*. New York: Chapman and Hall, 378–398.
- Harder LD, Barrett SCH.** 1993. Pollen removal from tristylous *Pontederia cordata*: effects of anther position and pollinator specialization. *Ecology* **74**: 1059–1072.
- Harder LD, Thomson JD.** 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* **133**: 323–344.
- Herrera CM.** 1993. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. *Ecological Monographs* **63**: 251–275.
- Johnston MO.** 1991. Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* **45**: 1468–1479.
- Kato M, Itino T, Hotta M, Inoue T.** 1991. Pollination of four sumatran *Impatiens* species by hawkmoths and bees. *Tropics* **1**: 59–73.
- Kerner A.** 1895. *The natural history of plants*. 2 Vols. Translated by FW Oliver. London: Blackie and Son.
- Knight SE, Waller DM.** 1987. Genetic consequences of outcrossing in the cleistogamous annual, *Impatiens capensis*. I. Population-genetic structure. *Evolution* **41**: 969–987.
- Lande R.** 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* **30**: 314–334.
- Lande R, Arnold SJ.** 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1026.
- Macior LW.** 1967. Pollen-foraging behavior of *Bombus* in relation to pollination of nototrobic flowers. *American Journal of Botany* **54**: 359–364.
- Macior LW.** 1982. Plant community and pollinatory dynamics in the evolution of pollination mechanisms in *Pedicularis* (Scrophulariaceae). In: Armstrong JA, Powell JM, Richards AJ, eds. *Pollination and evolution*. Royal Botanic Gardens, Sydney, 29–45.
- Manning A.** 1956. Some aspects of the foraging behavior of bumblebees. *Animal Behaviour* **9**: 164–201.
- Marden JH.** 1984. Intrapopulation variation in nectar secretion in *Impatiens capensis*. *Oecologia* **63**: 418–422.
- McCall C, Mitchell-Olds T, Waller DM.** 1989. Fitness consequences of outcrossing in *Impatiens capensis*: tests of the frequency-dependent and sib-competition models. *Evolution* **43**: 1075–1084.
- Mitchell RJ, Waser NM.** 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology* **73**: 633–638.
- Mitchell-Olds T, Shaw RG.** 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.
- Müller H.** 1883. *The fertilization of flowers*. Translated by D. Thompson. London: Macmillan.
- Murcia C.** 1990. Effects of floral morphology and temperature on pollen receipt and reward in *Ipomoea trichocarpa*. *Ecology* **71**: 1098–1109.
- Nilsson LA.** 1988. The evolution of flowers with deep corolla tubes. *Nature* **334**: 147–149.
- Ornduff R.** 1969. Reproductive biology in relation to systematics. *Taxon* **18**: 121–133.
- Percival M, Morgan P.** 1965. Observations on the floral biology of *Digitalis* species. *New Phytologist* **64**: 1–22.
- Pickens AL.** 1927. Unique method of pollination by the ruby-throat. *Auk* **44**: 24–27.
- Randall JL, Hilu KW.** 1990. Interference through improper pollen transfer in mixed strands of *Impatiens capensis* and *I. pallida*. (Balsaminaceae). *American Journal of Botany* **77**: 939–944.
- Reeve HK, Sherman PW.** 1993. Adaptation and the goals of evolutionary research. *Quarterly Review of Biology* **68**: 1–32.
- Rust RW.** 1977a. Pollination in *Impatiens capensis* and *Impatiens pallida* (Balsaminaceae). *Bulletin of the Torrey Botanical Club* **104**: 361–367.
- Rust RW.** 1977b. Pollinator service in sympatric species of jewelweed (*Impatiens*: Balsaminaceae). *Journal of the New York Entomological Society* **85**: 234–239.
- Schemske DW.** 1978. Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae): the significance of cleistogamy and chasmogamy. *Ecology* **59**: 596–613.
- Schemske DW.** 1984. Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. *Evolution* **38**: 817–832.
- Schmitt J, Ehrhardt D, Swartz D.** 1985. Differential dispersal of self-fertilized and outcrossed progeny in jewelweed (*Impatiens capensis*). *American Naturalist* **126**: 570–575.
- Schmitt J, 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.
- Simpson GG. 1953.** *The major features of evolution*. New York: Columbia University Press.
- Sprenkel CK, 1793.** *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen*. Berlin. Translated by P. Haase. Discovery of the secret of nature in the structure and fertilization of flowers. In: Lloyd DG, Barrett SCH, eds. *Floral Biology*. New York: Chapman and Hall, in press.
- Stanton ML, Asman TL, Galloway LF, Young HJ. 1992.** Estimating male fitness of plants in natural populations. In: Wyatt R, ed. *Ecology and evolution of plant reproduction*. New York: Chapman & Hall, 62–90.
- Stebbins GL. 1974.** *Flowering plants: evolution above the species level*. Cambridge, MA: Harvard University Press.
- Stewart SC, Schoen DJ. 1987.** Pattern of phenotypic viability and fecundity selection in a natural population of *Impatiens pallida*. *Evolution* **41**: 1290–1301.
- Straw RM. 1956.** Adaptive morphology of the *Penstemon* flower. *Phytomorphology* **6**: 112–119.
- Stuessy TF, Spooner DM, Evans KA. 1986.** Adaptive significance of ray corollas in *Helianthus grosseserratus* (Compositae). *American Midland Naturalist* **115**: 191–197.
- Takhtajan A. 1969.** *Flowering plants: origin and dispersal*. Translated by C Jeffrey. Washington, DC: Smithsonian Institution.
- Thompson JN. 1982.** *Interation and coevolution*. New York: Wiley.
- Thompson JN. 1988.** Variation in interspecific interactions. *Annual Review of Ecology and Systematics* **19**, 65–87.
- Thomson JD. 1981.** Field measures of flower constancy in bumblebees. *American Midland Naturalist* **105**, 377–380.
- Turesson G. 1922.** The genotypic response of the plant species to the habitat. *Hereditas* **3**: 211–350.
- von Frisch K. 1967.** *The dance language and orientation of bees*. Cambridge, MA: University Press Harvard.
- Wade MJ, Kalisz S. 1990.** The causes of natural selection. *Evolution* **44**: 1947–1955.
- Waser NM, Price MV. 1983.** Pollinator behavior and natural selection for flower color in *Delphinium nelsonii*. *Nature* **302**: 422–424.
- Weis AE, Abrahamson WG, Andersen MC. 1992.** Variable selection on *Eurosta's* gall size, I: the extent and nature of variation in phenotypic selection. *Evolution* **46**: 1674–1697.
- West-Eberhard MJ. 1983.** Sexual selection, social competition, and speciation. *Quarterly Review of Biology* **58**: 155–183.
- Wilson P.** In press. Pollination in *Drosera tracyi*: selection is strongest when resources are intermediate. *Evolutionary Ecology*.
- Wilson P, Thomson JD. 1991.** Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* **72**: 1503–1507.
- Wilson P, Thomson JD.** In press. How do flowers diverge? In: Lloyd D, Barrett SCH, eds. *Floral Biology*. New York: Chapman and Hall.
- Wilson P, Thomson JD, Stanton ML, Rigney LP. 1994.** Beyond floral Batemanian: gender biases in selection for pollination success. *American Naturalist* **143**: 283–296.
- Wolf LL, Stiles FG. 1989.** Adaptations for the 'fail-safe' pollination of specialized ornithophilous flowers. *American Midland naturalist* **121**: 1–10.
- Wood CE. 1975.** The Balsaminaceae in the southeastern United States. *Journal of the Arnold Arboretum* **56**: 413–426.
- Young HJ, Stanton ML. 1990.** Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **71**: 536–547.

