

Manipulation of Bee Behavior by Inflorescence Architecture and Its Consequences for Plant Mating

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ABSTRACT: Angiosperms display flowers in many three-dimensional arrangements, but the functional significance of this diversity is largely unknown. We examined influences of inflorescence architecture on pollination and mating by quantifying the responses of bumblebees to three architectures and then using these observations as the basis of a model that simulated pollen dispersal. On artificial panicles, racemes, and umbels, each with 12 identical flowers, bees visited one more flower, on average, on umbels than on panicles (with racemes being intermediate). In contrast to this weak response, the consistency of foraging paths among flowers differed strongly among architectures (raceme > panicle > umbel). The simulation model revealed limited differences in self-pollination and pollen export among the three inflorescence designs when all flowers presented and received pollen, because mating differences depended on only the number of flowers visited. In contrast, in simulations of inflorescences on which pollen receipt and presentation were segregated so as to minimize interference among flowers, the consistency of movement paths governed mating. In this case, racemes self-pollinated much less than umbels (with panicles being intermediate), and racemes exported much more pollen than umbels and panicles. These effects have diverse consequences for the evolution of inflorescence architecture, flower design, and sexual segregation.

Keywords: *Bombus*, bumblebee, dichogamy, monoecy, pollen export, self-pollination.

Thus the study of flower-branching and arrangement, which at best is perhaps not a particularly attractive subject, remains duller than it otherwise might be through this “cut and dried”

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manner of treatment. Hence an effort is here made to give these “dry bones” of descriptive morphology “flesh,” or at any rate some semblance of “flesh.” (Parkin 1914, p. 512)

Angiosperms exhibit extensive diversity in floral displays, typically presenting their flowers in aggregate structures (inflorescences) that differ considerably in their three-dimensional structure, or architecture (Troll 1964; Weberling 1989). This diversity arose through the evolution of alternative inflorescence architectures within and among lineages (e.g., Grimes 1999; Henderson 2002), which may occur readily, because inflorescence topology can change considerably with relatively simple developmental changes (Evans and Barton 1997; Doust and Kellogg 2002). Unrelated taxa also commonly exhibit convergent evolution of inflorescence architecture, suggesting that functional aspects of inflorescence architecture are subject to natural selection (Parkin 1914; Stebbins 1973). Nevertheless, the function of inflorescence architecture has received little experimental or theoretical analysis, despite Parkin’s (1914) initial attempt almost a century ago, so inflorescence diversity awaits explanation.

Hypotheses on the function of inflorescence architecture consider four aspects of reproduction. First, Wyatt (1982) proposed that inflorescence architecture affects the behavior of pollen vectors, thereby influencing pollen dispersal. For animal-pollinated plants, such effects could involve differences in pollinator attraction and/or pollinator behavior within inflorescences. For example, repeated transitions between condensed and elongate inflorescences in palms (Arecaceae) correlate with switches between beetle pollination on the one hand and bee, fly, or wasp pollination on the other (Henderson 2002). Second, Burt (1961) considered the advantages of aggregated heads of flowers, such as those that characterize the Asteraceae, and hypothesized that the production of many flowers with single ovules in a head increases mate diversity. Third, Stebbins (1973) and Wyatt (1982) suggested that inflorescence architecture influences competition among developing fruits, because architectures differ in their ability to distribute resources. Finally, Wyatt (1982) suggested that the three-dimensional arrangement of fruits might affect

the incidence of fruit and seed predation. These hypotheses address different aspects of reproduction and so consider different features of inflorescence design. In particular, inflorescence architecture can influence pollination only through the three-dimensional arrangement of flowers at any instant (“display architecture”), whereas any effect on resource allocation and fruit predation depends on the arrangement of all flowers (and fruits; “total architecture”). In addition, the optimal design of each inflorescence may be influenced by the number and arrangement of all inflorescences on a plant when they do not function independently (Schoen and Dubuc 1990; Fishbein and Venable 1996).

Unlike the effects of the number of flowers displayed by inflorescences on pollinator behavior and plant mating, which have been studied extensively (reviewed by Harder et al. 2001, 2004), few studies have tested the effects of inflorescence architecture on pollinator behavior, and none has examined its influence on plant mating. In the only study to address inflorescence architecture explicitly, Hainsworth et al. (1983) found that four hummingbirds visited and revisited fewer flowers on three-dimensional inflorescences than on one-dimensional inflorescences, in association with increases in foraging costs. Comparison of several studies of bumblebees visiting plants with different inflorescences indicates that inflorescence architecture influences the probability that a bee departs an inflorescence, which determines how many flowers were visited. For instance, bees commonly depart racemes after encountering a single flower containing less nectar than a threshold volume (Hodges 1985; reviewed in Kadmon and Shmida 1992), whereas bees typically do not leave capitate inflorescences (Cresswell 1990) or cymes (Kadmon and Shmida 1992) until they encounter several empty flowers. Such disparity between departure rules likely results from differences in the predictability of a bee’s expected rewards (Kadmon and Shmida 1992), which may be influenced by an architecture’s effect on movement paths. Collectively, these results reveal that inflorescence architecture provides a mechanism by which plants could manipulate pollinator foraging.

Any influence of inflorescence architecture on the number of flowers visited per pollinator should have diverse effects on plant mating. Self-pollination between flowers (geitonogamy) generally increases as each pollinator visits more flowers (e.g., Harder and Barrett 1995; Rademaker and de Jong 1998; Karron et al. 2004). Because geitonogamy involves the same processes as cross-pollination (Lloyd 1992), each self-pollen grain reduces the pollen exported to other plants (pollen discounting) so that increases in the number of flowers visited per pollinator can diminish a plant’s outcross siring success (Harder and Barrett 1995). The number of flowers visited per pollinator

can also affect each pollinator’s contribution to mate diversity through two effects. Pollinators that visit more flowers remove more pollen from each plant, increasing the number of plants that subsequently receive donor pollen. However, this effect is counteracted by increased depletion of the donor pollen on a pollinator by each recipient plant as pollinators visit more flowers per plant. Consequently, the specific effect of inflorescence architecture on a plant’s mate diversity will depend on the relative contributions of these competing influences. In addition, the probability that two pollen grains received by a stigma or plant share the same father (correlated mating; Ritland 1989) increases as relatively more pollen is received from each of a few mates (Harder and Barrett 1996). Correlated mating affects the opportunity for local mate competition, competition among full-sibling embryos for maternal resources, and selective development of embryos based on genetic quality. These mating characteristics also depend on floral traits that determine the amount of pollen that a pollinator removes from a flower and deposits on its stigma (Harder and Barrett 1996), which could modify the influences of inflorescence architecture on mating outcomes, including which architecture is most beneficial in a particular pollination environment.

The relative merits of different architectures may be enhanced if they differ in their ability to control the routes followed by pollinators among flowers when female and male function are segregated among different flowers. Sexual segregation occurs in about 50% of flowering plants (including both dichogamy and monoecy; Bertin 1993) and reduces geitonogamy and pollen discounting, particularly when pollinators consistently visit “male” flowers after “female” flowers (Harder et al. 2000; Routley and Husband 2003). All published demonstrations of the effectiveness of sexual segregation have involved plants with vertical inflorescences on which bees usually forage from bottom to top flowers. In this situation, presentation of male flowers above female flowers greatly reduces geitonogamy and increases pollen export per flower (Harder et al. 2000; Routley and Husband 2003). Whether pollinators follow consistent foraging paths on more complex and/or less vertical inflorescences remains to be examined, as does the effectiveness of sexual segregation on such inflorescences.

In this article, we describe two analyses of the effects of inflorescence architecture on pollinator behavior and plant mating. First, we examine the responses of bumblebees (*Bombus* spp.) to three inflorescence types: a vertical unbranched structure (raceme), a vertical branched structure (panicle), and a horizontal circular array (umbel). To eliminate differences among inflorescence types other than the three-dimensional arrangement of flowers, we used artificial inflorescences that presented 12 identical artificial flowers and equal nectar volumes. Our analysis of bee

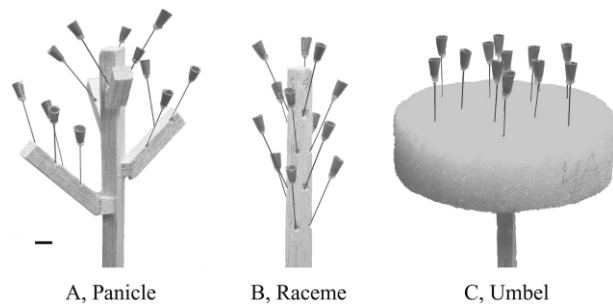


Figure 1: Examples of the artificial panicles (A), racemes (B), and umbels (C) used to study the effects of inflorescence architecture on foraging behavior by bumblebees. The scale bar represents 1 cm.

responses focuses on the number of flowers visited per inflorescence and the consistency of movement within inflorescences. We then used the recorded bee movements in a model simulating plant mating, which incorporates a range of parameter values that determine the exchange of pollen between flowers and bees. This model is applied twice: once simulating inflorescences with hermaphroditic flowers and once with the sex roles segregated among flowers within inflorescences. These simulations assess the likely contributions of inflorescence architecture to the reproductive performance of bee-pollinated plants and the extent to which pollination could influence the evolution of inflorescence diversity.

Methods

Inflorescence Types and Bee Behavior

We examined bee foraging on three types of artificial inflorescences, each with 12 flowers: a panicle, a raceme, and an umbel (fig. 1). The panicle had flowers along four branches of varying length (four, three, three, and two flowers). The raceme consisted of four whorls of three flowers arranged along a vertical stalk. The umbel presented flowers horizontally, with an outer ring of six flowers surrounding an inner ring of five flowers and a single central flower.

Each inflorescence consisted of artificial flowers mounted on either a balsa wood (panicle and raceme) or a Styrofoam (umbel) frame. A wooden pedicel supported each umbel to make the average elevation of flowers comparable among inflorescence types. We constructed each flower by gluing 1 cm of the closed end of a 1-mL centrifuge tube (painted blue) to the head of an insect pin, which served as a stalk. Immediately before presenting inflorescences to a bee, we filled flowers with 1 μ L of 40% sucrose solution using either a Hamilton dispensing syringe or an Eppendorf 1- μ L repeater pipette. During training and experimental trials, in-

dividual bees foraged on five inflorescences of one type, arranged linearly with approximately 25 cm between inflorescences in a 60 \times 150 \times 75-cm flight cage. Panicles were oriented with their branches in the same direction.

We recorded the responses to the three inflorescence types by workers from captive colonies of *Bombus huntii* Greene (four bees), *Bombus impatiens* Cresson (five bees), and *Bombus occidentalis* Greene (five bees). The *B. huntii* and *B. occidentalis* colonies were reared in the laboratory from queens caught in southern Alberta according to the procedures of Plowright and Jay (1966), as modified by Pomeroy and Plowright (1980). The *B. impatiens* colony was purchased from BioBest (Leamington, Ontario).

We trained individually marked bees to collect nectar from artificial inflorescences in the flight cage. During the first stage of training, bees encountered arrays of flowers on the floor of the flight cage, and individuals that foraged purposefully were chosen as subjects for subsequent trials. A bee was used in experiments once she collected nectar and delivered it to the colony quickly and repeatedly (approximately two to three foraging bouts per 30 min) during an extended period (at least 1 h). Being interested in the behavior of experienced foragers, we allowed each bee to become familiar with a particular inflorescence type during three “training” bouts (15 inflorescence visits) before we recorded her behavior. Flowers were not cleaned between these bouts.

During an experimental trial, each bee experienced at least three more foraging bouts on each inflorescence type, during which we videotaped her behavior (“data bouts”). When possible, we collected all data from a bee during a single day, and trials from a single treatment rarely spanned 2 days. We collected data from one bee at a time and collected all data for a particular inflorescence type before introducing the bee to a new type. Each bee encountered the three inflorescence types in random order, and no two bees of the same species experienced the same sequence. We used clean flowers for all data bouts.

We recorded the order of visits and revisits to an inflorescence’s flower positions by observing the videotape at one-sixth speed. The details of inflorescence construction determined a flower’s position and assured its consistent assignment. We analyzed data from only a bee’s first visit to an inflorescence during a bout, because nectar depletion may alter a bee’s behavior independently of the effects of architecture.

Simulation of Pollen Dispersal

Simulation Details. We explored the effect of inflorescence architecture on mating with simulations that combined the observed movement patterns of bees foraging on inflorescences and a geometric decay model of pollen dis-

persal (Bateman 1947; as modified by Harder and Wilson 1998b). The decay model assumes that pollen on a pollinator's body acts as a completely mixed pool, so that all pollen grains on a pollinator have the same probability of being deposited on a flower's stigma. Specifically, a pollinator deposits

$$D_i = PR\rho(1 - \rho)^{i-1} \quad (1)$$

pollen grains that it removed from a donor flower on the stigma of the i th subsequently visited recipient flower, where P is the number of pollen grains on the donor flower, R is the proportion of those pollen grains removed by the pollinator, and ρ is the proportion of the pollinator's pollen load deposited on each stigma (deposition fraction). According to this model, all pollen removed from a donor flower eventually reaches stigmas. This model, or an exponential variant, is used commonly as the basis of theoretical analyses of pollen dispersal and plant mating (e.g., Bateman 1947; Crawford 1984; Galen and Rotenberry 1988; de Jong et al. 1992; Harder and Barrett 1996), even though it predicts slightly less extensive dispersal than is typical for animal-pollinated plants (Morris et al. 1994). Our simulations considered all combinations of $\rho = 0.05, 0.1, \text{ and } 0.15$ and $R = 0.2, 0.4, \text{ and } 0.6$. These values lie within the ranges of published estimates for ρ (0.015–0.47; de Jong et al. 1992; Morris et al. 1994) and R (0.2–0.8; Harder 1990) for comparable models of pollen dispersal. All simulations used $P = 220$ pollen grains.

Our simulation accounted for the numbers of pollen grains in each flower's anthers, on its stigma, and on the bee's body. A bee removed entire pollen grains from individual flowers; however, it deposited fractional pollen grains on recipient stigmas, depicting expected dispersal for a given number of initial donor grains. We tracked pollen from each donor flower on a bee until <0.5 grains remained on the bee's body from that flower. For each flower visit, the simulation updated the number of pollen grains from each donor inflorescence on the flower's stigma, the number of pollen grains remaining in the flower's anthers, and the amount of pollen remaining on the bee's body from each previous donor flower, and it recorded which inflorescence donated the pollen. No pollen transferred between a flower's own anthers and stigma (autogamy), so that all mating outcomes result from pollen dispersal between flowers.

For each bee, we typically videotaped flower visits to 15 inflorescences of each inflorescence type, which served as the basis for a simulated bee's pollen dispersal. The simulation selected visits to inflorescences randomly with replacement to create a series of 100 inflorescence visits. Each simulated bee "experienced" the 100 inflorescences twice; the first sequence established an equilibrium of pollen on

the bee's body, and the second sequence provided the observations of pollen dispersal that we recorded. This process was repeated separately for each bee on each inflorescence type.

We ran two sets of simulations to examine the mating consequences of segregation of the sex roles among flower positions. For trials that simulated hermaphroditic flowers, all flowers acted simultaneously as pollen donors and recipients. For sexually segregated trials, the six flower positions at which bees usually commenced foraging on an inflorescence type ("start" positions) were assigned as female flowers. Male flowers were assigned to the remaining six "nonstart" positions. Given these arrangements, bees should tend to visit female flowers before male flowers within inflorescences, enhancing pollen import and export and reducing self-pollination among flowers. Comparison of results between the two sets of simulations permits assessment of interacting effects of sexual segregation and inflorescence architecture on mating.

Sexual segregation could affect mating in our simulations by two mechanisms. First, half of a segregated inflorescence's flowers are female and half are male, which reduces the opportunity for self-pollination and pollen export. Second, the tendency of pollinators to visit female flowers before male flowers governs the incidence of self-pollination and the associated pollen discounting. To distinguish between these effects, we calculated the female selfing rate and pollen export expected when pollinators visit V flowers, of which half are female and half are male, in random order for 1,000 inflorescences. Deviation from these random expectations in simulations that incorporate observed bee behavior identifies the effect of nonrandom foraging paths on mating. In addition, we present pollen export per pollen-presenting flower, rather than per inflorescence, to compare the hermaphroditic and sexually segregated simulations, because the segregated inflorescences present half as much pollen as those with hermaphroditic flowers.

Mating Parameters. We examined the effects of inflorescence architecture on four mating characters. Female selfing rate is the proportion of the pollen grains received by an inflorescence that were transferred between that inflorescence's flowers (geitonogamy). Pollen export is the number of pollen grains dispersed from an inflorescence to other inflorescences. The number of mates is the number of plants that donated pollen to an inflorescence. Any donor that provided more than one grain was counted as a full mate, whereas donors that provided less than one pollen grain were considered fractional mates. We then summed the mates and truncated this sum to an integer to ensure that an inflorescence does not have a fractional number of mates but also to allow fractional mates to

contribute to the number of mates. Finally, correlated outcrossing represents the proportion of all pairs of pollen grains imported by an inflorescence that are full sibs (following Ritland 1989). We examined the correlated outcrossing for the m inflorescences that contributed pollen to a particular inflorescence,

$$\frac{\sum_{k=1}^m n_k(n_k - 1)}{N(N - 1)}$$

(Harder and Barrett 1996), where n_k is the number of grains contributed by the k th father and N is the total number of grains received. We did not round pollen to integer grains; however, we considered only pollen from donors that contributed at least one pollen grain. We excluded visits to two inflorescences on which bees visited only flowers in nonstart positions from the calculation of female mating parameters (selfing rate, numbers of mates, and correlated outcrossing), because female flowers were not visited.

Data Analysis

We compared the number of flowers visited among inflorescence types and bee species with a two-factor, repeated-measures ANOVA (Mixed procedure of SAS version 8.2; SAS Institute 2001). This analysis considered the mean number of flowers visited by a bee on each architecture as individual observations and used restricted maximum likelihood (Jennrich and Schluchter 1986) to characterize the covariance among responses by individual bees. A model of heterogeneous compound symmetry was more appropriate than one of independent responses ($G = 20.31$, $df = 3$, $p < .001$). Denominator degrees of freedom for F -tests in these repeated-measures analyses were calculated by Satterthwaite's approximation (Littell et al. 1996), which can result in fractional degrees of freedom. We used Tukey's multiple comparisons to distinguish species and/or treatment means that differed significantly ($\alpha = 0.05$; Neter et al. 1996). In addition, we used a linear contrast (Kirk 1995) to test whether differences in the mean number of flowers visited varied linearly among species in relation to mean body mass, because large bees tend to visit more flowers per inflorescence (Rasheed and Harder 1997).

We compared foraging paths and the frequency of revisits to flowers among inflorescence types with generalized linear models (McCullagh and Nelder 1989; Genmod procedure of SAS version 8.2; SAS Institute 2001). The analysis of foraging paths considered the proportional use of start and nonstart flowers, a binomial dependent variable, which we analyzed after logit transformation. In con-

trast, the analysis of revisits considered a Poisson error distribution and ln transformation. These analyses accounted for repeated measurement of individual bees by the use of generalized estimating equations (Liang and Zeger 1986) and a variance-covariance model of compound symmetry. Tests of effects involved the generalized score statistic (T), which follows the χ^2 distribution (Rotnitzky and Jewell 1990; Boos 1992).

We analyzed mating outcomes from our simulations graphically rather than statistically for two reasons. First, the simulations drew inflorescence visits with replacement from a limited pool of observations, so that observations are not independent of each other. Second, each mating characteristic largely represents a transformation of the original bee behavior (e.g., see eqq. [2], [3]), so that tests of different mating characteristics are not independent of each other or of tests of bee behavior. The means and standard errors that we plot to illustrate mating outcomes were calculated from the average outcomes for each bee's simulation.

Results

Bee Behavior

Number of Flowers Visited. Overall, bees visited about two-thirds of the flowers on all inflorescence types, including occasional revisits (fig. 2). Bees visited significantly more flowers on umbels than on panicles, with racemes being intermediate and not differing significantly from either panicles or umbels ($F = 5.27$, $df = 2, 19.6$, $p < .05$; fig. 2A). On the basis of a general comparison, differences among bee species in the mean number of flowers visited per inflorescence were not quite significant ($F = 3.84$, $df = 2, 11$, $p = .054$); however, a test of the more specific hypothesis that the number of flowers visited differed among species in proportion to their mean body mass was significant ($F = 7.61$, $df = 1, 11$, $p < .025$; fig. 2B). The three species responded similarly to the three inflorescence types (architecture \times species interaction, $F = 1.96$, $df = 4, 19.6$, $p > .1$).

Bees seldom revisited flowers on experimental inflorescences (1% of flower visits and 8.7% of inflorescence visits). Architecture ($T = 2.98$, $df = 2$, $p > .2$), bee species ($T = 5.21$, $df = 2$, $p > .05$), and their interaction ($T = 5.69$, $df = 4$, $p > .2$) did not affect the number of revisits per inflorescence significantly. Revisits increased as bees visited more flowers on an inflorescence ($T = 7.33$, $df = 1$, $p < .01$; partial regression coefficient \pm SE = 0.339 ± 0.077). Overall, bees left 2.4% of inflorescences immediately after revisiting a flower, with no difference between architectures ($T = 4.57$, $df = 2$, $p > .1$). This percentage represents only 28% of the inflorescence visits

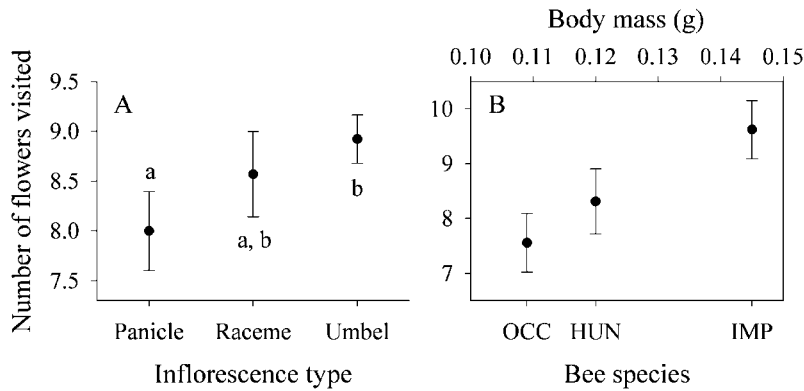


Figure 2: Mean (\pm SE) number of flowers visited by bees on artificial inflorescences. *A*, Comparison of visits to panicles, racemes, and umbels. *B*, Comparison of visits by *Bombus huntii* (HUN), *Bombus impatiens* (IMP), and *Bombus occidentalis* (OCC). In *A*, means that share the same letter do not differ significantly ($p < .05$), on the basis of Tukey multiple comparisons.

on which bees revisited flowers, so revisits to flowers did not typically motivate departure.

Foraging Paths. Inflorescence type strongly affected bees' foraging paths, as represented by the order of start and nonstart flowers within a visit sequence. Bees usually began visiting inflorescences (start positions) on the bottom six flowers on racemes, the outer six flowers on umbels, and the basal three flowers of the two lower branches on panicles ($\geq 94\%$ of visits to all architectures; table 1). In contrast, the tendency of bees to depart from nonstart positions differed significantly among inflorescence architectures (table 1), because they left from nonstart positions during 45% of their departures from umbels but about three-quarters of their departures from panicles and racemes. Before describing differences among inflorescence architectures in the use of start and nonstart flowers, we summarize the typical foraging paths by bumblebees on each of the three inflorescence architectures.

On racemes, bees usually started on the bottom whorl of three flowers (85% of arrivals) and then generally moved upward before leaving from flowers in the upper whorl (58% of departures). Bees moved upward during 71.0% of 1,119 movements between whorls (lower SE [LSE] = 0.96%, upper SE [USE] = 0.95%), which differed significantly from an equal frequency of upward and downward movements ($T = 371.36$, $df = 1$, $p < .001$; accounting for repeated measurement).

On panicles, bees almost always began foraging on the bottom two branches (98.2%) and generally moved up toward nonstart positions. Bees typically moved between adjacent flowers on the same side of a branch, which were closer to each other than to flowers on either the opposite side of the branch or other branches (see fig. 1). Overall,

bees moved upward along the inclined branches or between lower and upper branches significantly more often (59.6%, LSE = 1.08%, USE = 1.07%) than downward ($T = 76.72$, $df = 1$, $p < .001$; accounting for repeated measurement). This tendency for upward movement did not differ for movements along a branch or between branches ($T = 1.37$, $df = 1$, $p > .2$). To move from a lower to an upper branch, bees typically left the lower branch from the second-most basal flower, which was closer to flowers on upper branches than were more distal flowers. Bees usually departed panicles from one of the four distal flowers on the upper branches (55.5% of departures), although the distal flower on the longest lower branch was also a common departure point (10.2%).

On umbels, bees consistently began foraging on an outer flower (97.8% of visits) and moved quickly to the inner ring (table 1). Overall, a bee on an outer (start) flower was more likely to move to an inner (nonstart) flower (56.1%, LSE = 1.53%, USE = 1.52%) than to another outer flower ($T = 15.66$, $df = 1$, $p < .001$; accounting for repeated measurement), probably because inner flowers were closer than adjacent outer flowers (see fig. 1). In contrast, bees on an inner flower moved to an outer flower (52.6%, LSE = 1.39%, USE = 1.39%) or another inner flower (47.4%) with equal frequency ($T = 3.36$, $df = 1$, $p > .05$). Bees were more likely to depart umbels from outer (start) positions than from inner (nonstart) positions (table 1; $T = 13.78$, $df = 1$, $p < .001$).

The overall proportions of visits to start versus nonstart positions differed significantly among both inflorescence types (table 1) and bee species ($T = 7.35$, $df = 2$, $p < .05$). Start positions comprised approximately half of the flowers visited by bees for all three architectures, although bees visited relatively more start positions on panicles than

Table 1: Summary of bumblebee behavior on artificial panicles, racemes, and umbels

	Panicle	Raceme	Umbel	Test statistic (<i>T</i> ; <i>df</i> = 2)
Number of inflorescences	225	226	217	
Begin on a start position				1.785
Mean proportion of inflorescences	.948	.962	.978	
Lower SE	.027	.019	.010	
Upper SE	.018	.013	.007	
Visit a nonstart flower after beginning on a start flower				12.49**
Mean proportion of inflorescences	.464 ^A	.074 ^B	.824 ^C	
Lower SE	.040	.016	.029	
Upper SE	.040	.020	.026	
Leave from a nonstart position				11.42**
Mean proportion of inflorescences	.716 ^A	.791 ^A	.446 ^B	
Lower SE	.023	.032	.014	
Upper SE	.022	.028	.015	
Visits to start positions				8.56*
Mean proportion of inflorescences	.529 ^A	.517 ^{AB}	.503 ^B	
Lower SE	.008	.012	.007	
Upper SE	.007	.012	.007	

Note: Superscript letters indicate the outcomes of Šidák's multiple comparisons; inflorescence types with the same letter do not differ significantly ($\alpha = 0.05$).

* $p < .05$.

** $p < .01$.

on umbels, with racemes not differing significantly from either extreme (table 1). On panicles, bees also visited fewer nonstart than start flowers ($T = 14.54$, $df = 1$, $p < .001$). *Bombus occidentalis* visited a higher proportion of start positions (mean = 0.561, LSE = 0.011, USE = 0.012) than *Bombus impatiens* (mean = 0.492, LSE = 0.005, USE = 0.005; $T = 7.20$, $df = 1$, $p < .05$) and *Bombus huntii* (mean = 0.489, LSE = 0.008, USE = 0.008; $T = 6.29$, $df = 1$, $p < .05$), but *B. huntii* and *B. impatiens* visited similar proportions of start positions ($T = 0.14$, $df = 1$, $p > .5$). Architecture and bee species did not interact to affect visitation to the two functional positions ($T = 5.52$, $df = 4$, $p > .2$). *Bombus huntii* and *B. impatiens* visited start and nonstart positions in equal proportions ($p > .1$ in both cases), whereas *B. occidentalis* visited start positions more frequently than nonstart positions ($T = 27.84$, $df = 1$, $p < .001$).

Given the preceding results, it is not surprising that a bee's probability of visiting a nonstart flower changed at different rates as it visited successive flowers on different inflorescence architectures (table 2; architecture \times ln [sequence number] interaction; fig. 3). On racemes, this probability was initially low and increased steadily with successive visits (fig. 3, *solid line*; partial regression coefficient, $b \pm SE = 2.485 \pm 0.163$, $p < .001$). On panicles, bees also tended to visit several start flowers before moving to nonstart positions (fig. 3, *dashed line*; $b \pm SE = 0.931 \pm 0.188$, $p < .001$), although this tendency was not

as strong as on racemes ($T = 10.44$, $df = 1$, $p < .01$). In contrast, after bees arrived at umbels, they had a high probability of visiting a nonstart flower, which declined slowly with successive visits (fig. 3, *dotted line*; $b \pm SE = -1.128 \pm 0.167$, $p < .001$), so that the partial regression coefficient for ln(sequence number) for umbels differed significantly from that for panicles ($T = 9.96$, $df = 1$, $p < .01$) and racemes ($T = 12.04$, $df = 1$, $p < .01$).

Mating with Hermaphroditic Flowers

In our simulations of mating by inflorescences with simultaneously hermaphroditic flowers, the numbers of

Table 2: Analysis of a generalized linear model that evaluates the influences of bee species, inflorescence type, and a flower's position in a bee's sequence of visits within an inflorescence (ln transformed) on the probability of visiting a nonstart flower position

Source	Response	
	<i>T</i>	<i>df</i>
Species	5.04	2
Architecture	12.21**	2
Species \times architecture	5.49	4
ln(sequence number)	11.76***	1
Architecture \times ln(sequence number)	12.19**	2

** $p < .01$.

*** $p < .001$.

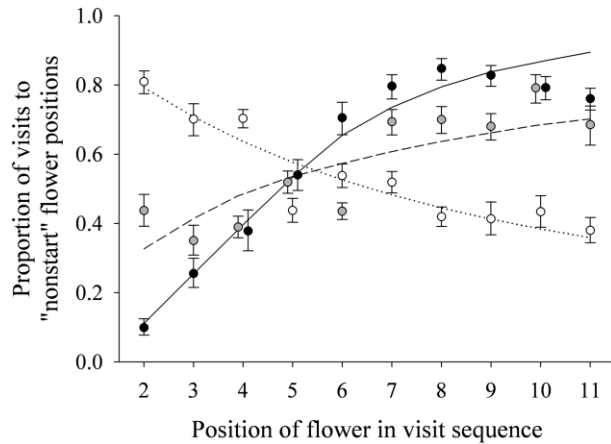


Figure 3: Mean proportion (\pm SE) of visits to a nonstart position as bees visit successive flowers on three inflorescence architectures: panicles (gray symbols, dashed line), racemes (solid symbols, solid line), and umbels (open symbols, dotted line). The prediction lines are based on the generalized linear model presented in table 2.

flowers visited and revisited completely determine the mating environment. For the geometric decay model, the female selfing rate resulting from a pollinator visiting V flowers is

$$S = 1 - \frac{1 - (1 - \rho)^V}{\rho V}, \quad (2)$$

if self-pollination occurs only between flowers (Harder and Barrett 1996). Accordingly, each additional flower visited on an inflorescence with hermaphroditic flowers increments overall geitonogamy, especially when each stigma removes a large fraction of the pollen on a bee's body (cf. ρ in fig. 4A). However, this increment diminishes with successive visits as pollen on a bee's body from early flowers is depleted. In contrast, the amount of pollen removed from anthers (PR) does not affect the selfing rate in the geometric decay model. The number of pollen grains exported to other plants,

$$X = \frac{PR(1 - [1 - \rho]^V)}{\rho} \quad (3)$$

(Harder and Barrett 1996), also increases in a decelerating manner with the number of flowers visited (fig. 4B). According to equation (3), self-deposited pollen reduces pollen export directly, so that export declines as the deposition fraction (ρ) increases (cf. gray symbols in fig. 4B). Equation (3) also proposes that pollen export increases directly with the amount of pollen removed per flower (PR). Our simulations of self-pollination and pollen export reproduce

the relations predicted by equations (2) and (3) (fig. 4; cf. lines and symbols), except when bees revisited flowers, which was always true when the number of visits to flowers exceeded the 12 available.

Inflorescence architecture weakly affected average mating by inflorescences with hermaphroditic flowers. The additional flower that bees visited on umbels compared with panicles (fig. 2A) caused slightly more geitonogamy (fig. 5A; cf. symbols of the same shape with different shading along the abscissa). Despite the pollen discounting associated with visits to more flowers, umbels also exported more pollen than panicles (fig. 5B), because bees removed more pollen during the additional flower visit than was lost to discounting. This export advantage for

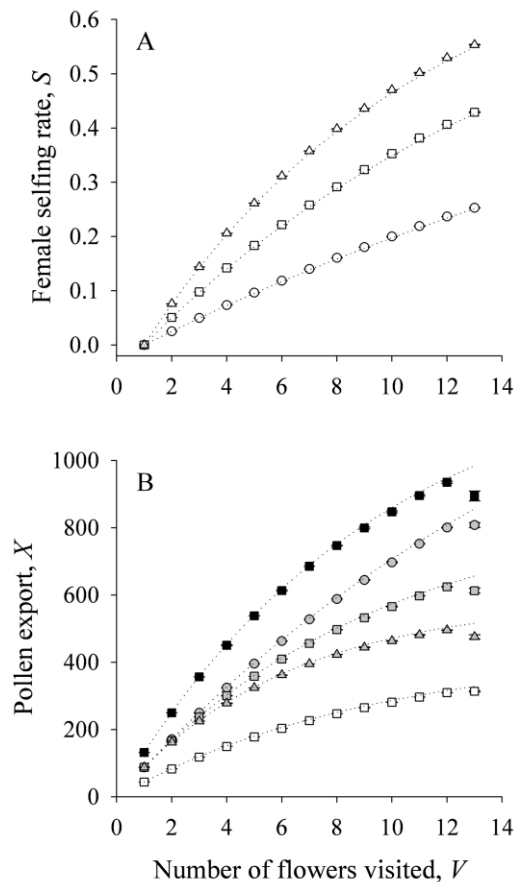


Figure 4: Relations of (A) female selfing rate (S) and (B) total pollen export (X) to the number of flowers visited on an inflorescence (V) for different intensities of pollen removal from anthers ($R = 0.2$, open symbols; $R = 0.4$, gray symbols; $R = 0.6$, solid symbols) and pollen deposition on stigmas ($\rho = 0.05$, circles; $\rho = 0.1$, squares; $\rho = 0.15$, triangles) for inflorescences with hermaphroditic flowers. Symbols depict mean (\pm SE) simulation results, whereas the dotted lines illustrate the relations described by equations (2) and (3).

Mating with Sexual Segregation

umbels would not persist if flowers received visits from enough pollinators to remove all of their pollen, in which case pollen discounting alone would affect differences among inflorescences in pollen export.

The composition of pollen received by plants varied negatively with the number of flowers that a bee visited. Umbels, on which bees visited the most flowers, typically received pollen from 0.4 to 1.5 fewer donor inflorescences than panicles on average, with racemes being intermediate (fig. 5C). When a bee visits many flowers per inflorescence, each plant receives a larger fraction of a bee's pollen load from a specific donor inflorescence, so fewer plants can receive the donor's pollen. In turn, correlated outcrossing varied negatively with the number of plants donating pollen to a recipient plant, because receipt of fewer grains per donor reduced the proportion of all pollen pairs that are full sibs. Consequently, umbels typically experienced 6.3%–7.8% greater correlated outcrossing than panicles, depending on R and ρ (fig. 5D).

Compared to simulations with hermaphroditic flowers, sexual segregation reduced self-pollination by up to 82%, 65%, and 41% for racemes, panicles, and umbels, respectively, with associated but smaller increases in pollen export per flower (60%, 37%, and 27%, respectively; fig. 5A, 5B; compare symbols of the same shape with different shading along the ordinate). The effects of sexual segregation on plant mating differed among architectures because of their contrasting influences on bee visitation to female and male flowers. Self-pollination by umbels did not differ from that expected with completely random pollinator movement among equal numbers of female and male flowers (fig. 5A; compare open symbols with the dotted line). In contrast, sexually segregated panicles and racemes experienced much less self-pollination than occurs when pollinators move randomly among flowers, because bees visited male flowers after female flowers quite

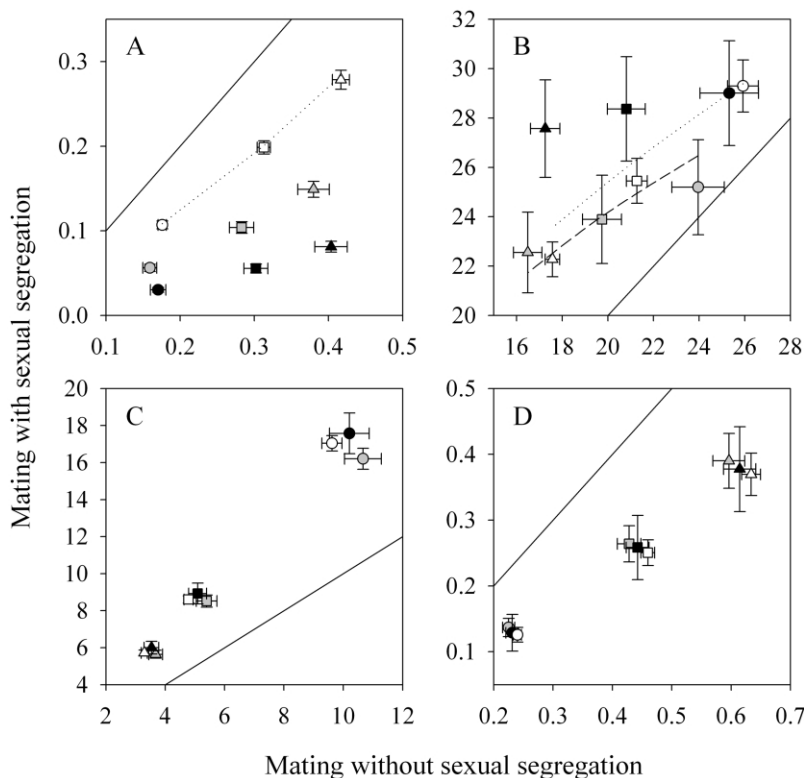


Figure 5: Comparison of mean (\pm SE) simulated mating with hermaphroditic flowers or sexual segregation based on the behavior of bumblebees visiting panicles (gray symbols), racemes (solid symbols), and umbels (open symbols) when bees remove 20% of the pollen available in anthers (i.e., $R = 0.2$). A, Female selfing rate. B, Pollen export per pollen-presenting flower. C, Number of mates. D, Correlated outcrossing. The different symbols identify the effects of stigmas removing different proportions of the pollen on pollinators' bodies ($\rho = 0.05$, circles; $\rho = 0.1$, squares; $\rho = 0.15$, triangles). The solid diagonal line in each plot depicts equal mating performance with hermaphroditic flowers or sexual segregation. The dashed and dotted lines in A and B depict the mating outcomes expected if bees move randomly during visits to eight or nine flowers (without revisits) within an inflorescence, respectively (the dashed line is not shown in A because it largely overlaps the dotted line).

consistently on racemes and, to a lesser extent, on panicles. Sexual segregation reduced the female selfing rates of panicles and racemes most strongly when stigmas removed a relatively large fraction of a bee's pollen load ($\rho = 0.1$ or 0.15 ; fig. 5A).

The limited geitonogamy on segregated racemes left more pollen on bees as they departed a donating plant, so that racemes also exported much more pollen than panicles or umbels when stigmas removed a relatively large fraction of a bee's pollen load ($\rho = 0.1$ or 0.15 ; fig. 5B). In contrast, pollen export by umbels and panicles did not differ from that expected with random pollinator movement among equal numbers of female and male flowers (fig. 5B; compare open symbols and dotted line for umbels and gray symbols and dashed line for panicles). This lack of improvement resulted from different causes: on umbels, bees visited female (start) and male (nonstart) flowers equally (table 1) but moved indiscriminately among positions (fig. 3), whereas on panicles, bees moved more consistently from female to male flowers (fig. 3) but visited fewer male than female flowers (table 1; $T = 14.54$, $df = 1$, $p < .001$).

The joint effects of inflorescence architecture and sexual segregation on pollen export depended on the proportion of pollen on bees' bodies removed by each stigma (ρ ; fig. 5B). With a small deposition fraction (i.e., $\rho = 0.05$; fig. 5B, circles), the extra pollen that bees removed from umbels by visiting an additional flower essentially balanced the associated pollen discounting, so umbels and racemes exported pollen roughly equally. In contrast, a large deposition fraction (i.e., $\rho = 0.15$; fig. 5B, triangles) aggravated pollen discounting more strongly for umbels, so that they exported much less pollen than racemes. These results illustrate interacting effects of inflorescence architecture and a floral trait (ρ) on plant mating when the sex roles are segregated among flowers. In contrast, pollen export varied in direct proportion to pollen removal (R), regardless of whether the sex roles were segregated (results not shown).

Compared with hermaphroditic flowers, sexual segregation increased mean mate number by at least 50% (fig. 5C) and reduced average correlated outcrossing by about 40% (fig. 5D) for all architectures, although it did not accentuate the overall differences among architectures for these mating characters. Mate diversity increased because sexual segregation halved the number of pollen-receiving flowers on each plant, so that bees visited more plants before depleting the pollen that they carried from a specific donor plant. Relative to hermaphroditic flowers, racemes replaced panicles as the architecture with the slightly greater number of mates when the sex roles were segregated. Architectures did not differ greatly in correlated outcrossing; however, sexual segregation reversed the rank-

ing of architectures for correlated outcrossing compared with hermaphroditic flowers, with panicles experiencing the most correlated outcrossing and umbels the least (fig. 5D).

Effects of Bee Species on Plant Mating

Foraging differences among the three bee species that we studied created variation in plant mating, which was largely unaffected by sexual segregation, because bee species differed weakly in the number of flowers visited per inflorescence (fig. 2B) but not in the consistency of their foraging paths (table 2). By visiting two more flowers per donor inflorescence, *B. impatiens* caused an average of 25% more self-pollination and exported 20% more pollen than *B. occidentalis*, with *B. huntii* being intermediate, over all values of ρ and R that we considered (hermaphroditic simulations only). Correspondingly, by visiting more flowers per recipient inflorescence with hermaphroditic flowers, *B. impatiens* carried donor pollen to an average of 22% fewer recipient plants than did *B. occidentalis*, which resulted in 19% higher correlated outcrossing on inflorescences visited by *B. impatiens*. Interestingly, sexual segregation reordered the species effects on mate diversity and correlated outcrossing, so that *B. occidentalis* carried donor pollen to an average of 17% fewer recipient plants than did *B. huntii*, which resulted in 16% higher correlated outcrossing on inflorescences visited by *B. occidentalis*.

Discussion

Inflorescence Architecture and Pollinator Behavior

Inflorescence architecture confronts pollinators with a complex foraging problem. This complexity is evident in the contrasting rankings among architectures in the number of flowers visited (fig. 2A) and the consistency of foraging paths within inflorescences (fig. 3).

The tendency of bees to visit more flowers on umbels than on panicles, with racemes being intermediate (fig. 2A), may partly reflect the closer spacing of flowers on umbels, which would reduce the cost of searching for an overlooked flower. However, the more three-dimensional structure of panicles also affects foraging costs, because bees took longer to fly the same distance between flowers on panicles than on racemes or umbels (results not shown; also see Hainsworth et al. 1983). Inflorescence complexity may also limit a bee's ability to identify the shortest foraging path that incorporates all flowers. Whatever the cause, inflorescence architecture had limited effects on the number of flowers that bees visited, although our experiment may underestimate this effect, because all flowers offered the same nectar volume. In contrast, in natural

situations nectar availability varies among flowers because of differences in the time since they were last visited and because, as described in the first section of this article, bees typically leave racemes after encountering fewer empty flowers than when they visit less structured inflorescences.

In our experiments, inflorescence architecture primarily influenced the consistency of bees' foraging paths (fig. 3). Of our inflorescences, racemes allowed the strongest expression of bumblebees' innate tendency to begin foraging low on vertical inflorescences and then move upward (reviewed by Harder et al. 2001), resulting in highly consistent foraging paths. Our panicles also have a vertical dimension, both within the inclined branches and between lower and upper branches (fig. 1), but their asymmetrical branched structure increases the variety of possible foraging paths, thereby complicating consistent expression of upward movement. In contrast, our umbels are flat and present a more uniform distribution of flowers, which greatly increased the number of possible foraging paths of equivalent length and cost. Consequently, bees moved essentially randomly between inner and outer flowers (also see Giurfa and Núñez 1993). The extent to which natural variation in nectar (or pollen) availability among flowers might additionally affect the consistency of foraging paths is unclear, because relevant data exist only for racemes. In this case, nectar gradients influence where bees commence foraging (Best and Bierzychudek 1982) but not their tendency to forage upward, regardless of whether upper or lower flowers offer more nectar (Waddington and Heinrich 1979).

Together, the contrasting responses by bees to our three inflorescence architectures indicate that plants can modify pollinator behavior through the three-dimensional arrangement of their flowers. In addition, inflorescence architecture may affect pollinator attraction by influencing the density of the floral display and its contrast with the surrounding environment. These effects and the influences of the number of open flowers on pollinator behavior (reviewed by Ohashi and Yahara 2001) provide many opportunities for selection to modify interactions between plants and their pollinators through characteristics of their floral displays.

Mating and the Evolution of Inflorescence Diversity

Our simulations revealed that inflorescence architecture can affect the character of mating for animal-pollinated plants and so can be subject to selection for improved mating success. Inflorescence architecture serves a pollination function only for outcrossing species. Such species typically carry enough genetic load that selfed offspring are less valuable than outcrossed offspring, so self-fertilization is often undesirable (Lande and Schemske

1985). In addition, pollen discounting resulting from geitonogamy reduces pollen export opportunities (reviewed by Harder and Wilson 1998a). Therefore, in the following discussion, we regard traits that favor low self-pollination and high pollen export to be most advantageous. We will not consider mate diversity and correlated outcrossing further, because they differed little between inflorescence architectures in our simulations (fig. 5C, 5D), suggesting that they play a lesser role in the selection of architecture (also see Mitchell et al. 2005). Instead, mate diversity and correlated outcrossing, which affect opportunities for competition between male gametophytes and developing embryos (Ritland 1989), probably evolve in concert with floral traits (see Harder and Barrett 1996) and sexual segregation (fig. 5C, 5D).

In our simulations, inflorescence architecture affected self-pollination and pollen export primarily when mating outcomes depended on the order in which pollinators visited flowers. When all flowers functioned simultaneously as pollen donors and recipients, the foraging path did not influence pollen dispersal, so the small differences in the number of flowers visited among architectures caused limited differences in mating characters. Instead, variation in mating outcomes in this situation resulted largely from differences in floral traits that control pollen exchange with pollinators (fig. 5). In contrast, sexual segregation greatly reduces interference between flowers within an inflorescence when pollinators visit pollen-receiving flowers before pollen-donating flowers, which occurred most consistently on racemes and least consistently on umbels. Note that sexual segregation enhances pollen export by the plant as a whole, which is most relevant to selection, only if it does not reduce a plant's overall pollen presentation. Instead, aggregate pollen presentation must be maintained either by all flowers serving female and male function at different stages of their lives, as with dichogamy, or by monoecious plants producing more flowers than hermaphroditic species.

The effectiveness of sexual segregation depends on the relation of pollinator behavior to inflorescence traits. When umbels cannot impose specific foraging routes on pollinators, as in our experiments, selection may favor a different pattern of sexual segregation. In particular, bee-pollinated species with umbels provide many examples of synchronous protandry, whereby flowers within an inflorescence proceed through male and then female phases simultaneously (e.g., Apiaceae: Molano-Flores 2001; Araliaceae: Thomson and Barrett 1981; Alstroemeriaceae: Aizen and Basilio 1995; Butomaceae: Bhardwaj and Eckert 2001), thereby restricting self-pollination. However, our umbels are not typical of the flat inflorescences of many species with umbels, capitula, or corymbs, because the flowers were spaced so that bees had to fly between flowers.

Species with denser, flat inflorescences on which pollinators walk among flowers, such as the umbels of many Apiaceae and capitula of most Asteraceae, commonly open flowers sequentially, with older, pollen-receiving flowers outside of younger, pollen-donating flowers (Burt 1977; Webb 1981). Because bees almost always began foraging on outer flowers on flat inflorescences (table 1), this pattern of sexual segregation could enhance mating if the arrangement of flowers causes pollinators to visit outer flowers and then inner flowers before they depart the inflorescence, in contrast to the inconsistent foraging patterns that we observed on umbels. Giurfa and Núñez's (1993) observations of random movement by honeybees on *Carduus acanthoides* capitula are inconsistent with this expectation. However, they studied inflorescences that had been bagged previously and so presented unusually abundant nectar. In contrast, Giurfa and Núñez mentioned unpublished observations by S. Vogel that bees move around the periphery of *C. acanthoides* inflorescences under more natural conditions.

Unlike umbels, our racemes and panicles allowed expression of innate upward movement patterns by bees, so sexual segregation can be implemented more effectively on such inflorescences. Racemes benefited most from sexual segregation in our simulations (fig. 5), whereas panicles realized less self-pollination than umbels (fig. 5A) but similar pollen export per flower (fig. 5B). This lack of an export advantage for panicles resulted from a mismatch between the proportions of female and male flowers and their relative visitation. Thus, adaptive implementation of sexual segregation involves both the distribution of female and male flowers within the inflorescence and their relative abundance.

The joint evolution of inflorescence architecture and sexual segregation is evident in the repeated evolution of andromonoecy in *Solanum* (see Symon 1979; Whalen and Costich 1986). The ancestral state in *Solanum* involves multibranched inflorescences with hermaphroditic flowers, whereas derived andromonoecious species have less branched inflorescences with basal hermaphroditic flowers and male flowers at the tips. Because pollen-collecting bees principally pollinate *Solanum* (reviewed by Symon 1979), the mating consequences of different patterns of sex expression within *Solanum* inflorescences likely resemble those of our simulations for panicles and racemes. These simulations suggest that sexual segregation evolves most readily on less branched inflorescences, because it promotes mating most strongly when bees can follow their proclivity to move upward, as long as the numbers of flowers serving different sex roles match the frequency of pollinator visits to different positions within the inflorescence (fig. 5).

Our simulations suggest that inflorescence architecture

sets the context for the selection of floral traits and vice versa, especially when sex function is segregated within inflorescences. Of particular relevance are floral traits that determine the duration and site of contact between the stigma and pollinator, thereby affecting the proportion of pollen that each stigma removes from a pollinator's body (ρ ; see Galen and Plowright 1985; Murcia 1990; Johnston 1991). In general, interference among flowers resulting from geitonogamous visits increases with ρ (fig. 4), so the strength of selection on traits that affect ρ probably depends on how inflorescence architecture influences the incidence of geitonogamy. Without sexual segregation, differences in ρ cause roughly equivalent changes in mating for all architectures (fig. 5). With sexual segregation, differences in ρ affect mating by umbels much more strongly than that of racemes (fig. 5), so that floral traits that govern pollen receipt may be under stronger selection on umbels. Similarly, the prevailing ρ probably affects the strength of selection on inflorescence architecture, because racemes outcross much more than panicles, or especially umbels (fig. 5), only when ρ is large and the sex roles are segregated. Consequently, evolutionary transitions from one inflorescence architecture to another may be more likely when stigmas remove a relatively large fraction of the pollen on a pollinator's body.

Mechanisms other than sexual segregation that reduce geitonogamy, such as heterostyly or enantiostyly (Jesson et al. 2003), may also evolve more readily in association with particular inflorescence architectures. For example, families in which heterostyly is particularly prevalent typically produce flowers in umbels or cymes (e.g., Linaceae, Oxalidaceae, Plumbaginaceae, Primulaceae, Rubiaceae). We have demonstrated that pollinators do not exhibit consistent movement patterns on such inflorescences and that sexual segregation is relatively ineffective. Consequently, an ancestral inflorescence architecture may predispose the evolution of particular floral mechanisms for promoting mating.

The most important aspect of inflorescence architecture for manipulating pollinator behavior seems to be the three-dimensional arrangement of flowers, whereas inflorescences are traditionally characterized by their branching patterns (Troll 1964; Weberling 1989). Simple changes in the relative lengths of inflorescence branches and/or flower pedicels can greatly modify the spatial arrangement of flowers without altering branching patterns. For example, the inflorescence of *Narcissus gaditanus* Boissier & Reuter is typologically an umbel, but a gradient in pedicel length allows flowers to be presented vertically, so that the inflorescence probably affects pollination like a raceme. Such flexibility in pedicel and branch lengths may facilitate selection for enhanced outcrossing without compromising other inflorescence functions, such as resource distribu-

tion, or fruit dispersal. In addition, this flexibility probably increases the opportunities for selection to modify inflorescence architecture, resulting in the extensive inflorescence diversity within angiosperms.

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