

## EVOLUTION OF INFLORESCENCE DESIGN: THEORY AND DATA

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**Abstract.**—Very low fruit set in milkweeds and other flowering plants often has been attributed to greater sexual selection on inflorescence size via male, rather than female, reproductive success. Although this explanation has been generally accepted, alternate explanations have been presented, and recently the “male function” or “pollen donation” hypothesis has been sharply criticized. In this paper, we make the distinction between selection on total flower number and on the size of inflorescence units, both of which have been termed “inflorescence size.” We present an ESS model for the evolution of inflorescence design that considers reproductive success through male and female function. The model predicts that selection will balance the proportional changes in female and male reproductive success resulting from changes in inflorescence-unit size. We conducted a field study of selection on the size of inflorescence units (umbels) by manipulating umbel size and number in a natural population of *Asclepias tuberosa*, in southeastern Arizona, during two reproductive seasons. We found that the male fitness function reached a maximum at an intermediate umbel size in both years (although not significantly different from the smallest umbel size in either year), whereas the female fitness function was highest for the smallest umbel size in one year, but was constant across umbel sizes in the other year. We also found that pollinator visitation rate corresponded well with male, but not female, function, and that between-year variation in the male reproductive success of different umbel sizes corresponded with variation in the composition of the pollinator pool. Our empirical results, when inserted in the model, predict ESS umbel sizes similar to those observed in the study population and the species throughout its range.

**Key words.**—*Asclepias tuberosa*, ESS model, inflorescence design, inflorescence size, milkweed, pollen transfer, sexual selection, size-number trade-offs.

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Willson and Rathcke's (1974) groundbreaking study of the adaptive design of milkweed inflorescences introduced the utility of considering separately fitness achieved through male and female function in cosexual plants (sensu Lloyd 1982). This distinction has contributed to the blossoming of theoretical plant reproductive biology (reviewed in Bertin 1988; Willson 1994), although some applications of this approach are controversial (Lyons et al. 1989; Wilson et al. 1994). Yet, there has been considerable disagreement about the conclusions drawn from the first empirical examination of this theory, the inflorescence design of milkweeds (Willson and Rathcke 1974; Willson and Price 1977). In particular, Wyatt, Broyles, and coworkers (Broyles and Wyatt 1990a, 1995; reviewed in Wyatt and Broyles 1994), also working with milkweed species, have called into question both the underlying assumptions and the interpretation of data bearing on the importance of selection through male function. The aims of the present paper are to clarify concepts surrounding the evolution of inflorescence design, to provide an analytical model for the evolution of inflorescence-unit size, and to evaluate the role of differential selection through male and female function on the evolution of milkweed inflorescences.

The floral displays of flowering plants are arranged hierarchically. For many plants, flowers are grouped into modular units, such as the umbels of milkweeds, that are reiterated in the development of the total floral display. Additionally, these basic units may be organized at intermediate levels, such as branches and stems in *Asclepias* (Wyatt 1980; Broyles and Wyatt 1995). We introduce the term “inflorescence design” to refer to the manner in which total flower number is apportioned between the size and number of inflorescence units. We use “inflorescence unit” to refer to the smallest level of aggregation of flowers in discrete structures that are replicated to comprise the total floral display. “Total floral

display” refers to all of the flowers on a plant. Because of imprecise definitions of “inflorescence” (Wyatt 1982), we avoid its unmodified use in this paper. “Inflorescence” has been used to refer to any level of organization of the floral display, from the inflorescence unit (e.g., Willson and Rathcke 1974; Bell 1985) to the total floral display (e.g., Broyles and Wyatt 1990a). Often, it refers to multiple levels (e.g., Cohen and Shmida 1993). This imprecision may be partly responsible for conflicting interpretations of the importance of male function in selection on the size of “inflorescences” (e.g., Willson and Rathcke 1974; Bell 1985; Broyles and Wyatt 1990a, 1995).

The necessity of distinguishing male and female function in cosexual plants has been demonstrated in many cases (e.g., Stanton et al. 1986). However, the importance of this distinction for understanding the evolution of inflorescence design has yet to be established convincingly. Sutherland (1986, 1987) enumerated several hypotheses that can account for the evolution of large floral displays, and has found some support for the relative importance of selection through male function in both comparative and population-level studies. Large floral displays have been thought to be selected primarily through male function because female reproductive success (RS) is often resource limited, putting an upper bound on the number of fruits that can be produced, whereas male RS may be limited by access to mates, giving increasing RS with size of floral display (i.e., Bateman's principles: Willson 1979; Queller 1983; Lloyd 1984; but see Wilson et al. 1994). This assumption may not hold, however, for plants that are chronically pollination limited (Broyles and Wyatt 1990a).

A theoretical model for the evolution of inflorescence design was presented by Schoen and Dubuc (1990). They considered mainly optimal inflorescence design of self-compatible and self-incompatible plants, emphasizing the mobility

of resources utilized for flower production. The principal result of their analysis is that the fitness-maximizing inflorescence-unit size depends on the degree of integration of stems or branches, in terms of resource allocation. Their analysis assumed that male and female fitnesses are fixed, frequency-independent functions of inflorescence design. However, these fitness functions have also been modeled as frequency-dependent functions of floral traits (e.g., Lloyd 1987). The empirical relationship between different levels of floral display (i.e., umbels, branches, stems) and male and female reproductive success has been reported by Wyatt (1980) and Broyles and Wyatt (1990a, 1995).

A significant impediment to progress in evaluating the importance of selection via male function is the difficulty of measuring male RS in natural populations (Stanton et al. 1992; Snow and Lewis 1993). Realized male fitness has been estimated most satisfactorily with genetic markers (Stanton et al. 1986; Broyles and Wyatt 1990a,b; Devlin et al. 1992; Snow and Lewis 1993). Because of the difficulty of measuring pollen movement directly, however, there are few documented measures of per visit pollen-removal rates (Young and Stanton 1990) or proportions (Harder 1990). Even so, male fitness components have been reported with respect to variation in several floral characters, such as flower size (Bell 1985; Young and Stanton 1990), pollen size (Harder 1990), pollen production (Young and Stanton 1990), size of total floral display (Bell 1985; Broyles and Wyatt 1990a,b; Devlin et al. 1992), and inflorescence-unit size (Willson and Price 1977; Chaplin and Walker 1982; Broyles and Wyatt 1995). Milkweed pollination is an excellent system for studying components of male RS because of the relative ease of quantifying pollen movement through pollinia (aggregated pollen grains that are transported as discrete units; see Wyatt and Broyles 1994; Fishbein and Venable 1996), and because pollinia removal rates have been shown to be correlated significantly with more comprehensive measures of male RS (Broyles and Wyatt 1990a).

A further impediment to understanding the evolution of inflorescence design has been an incomplete development of the theory of how selection operates simultaneously through male and female fitness components at different levels of the floral display. In this study, we present a model of how sexual selection determines the size and number of inflorescence units on a plant and examine empirically the roles of female and male function in the evolution of inflorescence design.

#### *A Model for the Evolution of Inflorescence Design Considering Male and Female Function Separately*

Consider a population of plants with hermaphroditic flowers arranged in some inflorescence design. Reproductive success (fitness) can be partitioned into a component due to the number of flowers per inflorescence unit and the number of these units. For simplicity, intermediate levels of arrangement (e.g., stems, branches) are not considered. Per flower RS can also be partitioned into male and female components. Thus, the RS of an individual plant can be expressed as

$$w(z, I) = f_{\varphi}(z)zI + f_{\delta}(z)zI, \quad (1)$$

where  $w(z, I)$  is the fitness of an individual with inflorescence-

unit size,  $z$ , and inflorescence-unit number,  $I$ ,  $f_{\varphi}(z)$  is the per flower RS through seed production (a function of inflorescence-unit size), and  $f_{\delta}(z)$  is the per flower RS through male function (also a function of inflorescence-unit size). Thus, the first term on the right side of equation (1) is the individual fitness through female function (i.e., number of seeds produced) and the second term is the fitness through male function (i.e., number of seeds sired on other plants).

The fitness of an individual in a sexual, outcrossing population depends not only on its own reproduction strategy, but also on that of others. The reproductive strategies of possible mates will determine the number of seeds an individual can potentially sire, whereas those possible competitors (i.e., other pollen parents) will determine the number of seeds that are actually sired.

Equation (1) can be rewritten more explicitly to account for this context dependence of individual fitness:

$$w(z', I', z, I) = f_{\varphi}(z')z'I' + \frac{Nf_{\varphi}(z)zI f_{\delta}(z')z'I'}{Nf_{\delta}(z)zI}, \quad (2)$$

where  $w(z', I', z, I)$  is the fitness of an individual with inflorescence-unit size and number,  $z'$ ,  $I'$ , in a population of individuals with inflorescence-unit size and number  $z$ ,  $I$ .  $N$  is the population size and  $f_{\delta}(z)$  is the per flower pollen-removal rate (a function of inflorescence-unit size). The first term on the right side of equation (2), the fitness through female function of an individual, is assumed to be a function only of its own inflorescence-unit size,  $z'$ , and number,  $I'$ , (this assumes no pollen limitation of seed production). The second term is an individual's fitness through male function. It equals the product of the number of seeds available for siring in the population,  $Nf_{\varphi}(z)zI$ , times the proportion of these sired by the individual in question,  $f_{\delta}(z')z'I'/Nf_{\delta}(z)zI$ . This formulation assumes that, on average, the proportion of seeds sired by an individual depends on the proportion of pollen grains removed from all flowers that is made up of its own pollen grains (i.e., no systematic postremoval bias in pollen transfer due to inflorescence-unit size). The denominator is expressed as  $Nf_{\delta}(z)zI$  instead of the more intuitive  $f_{\delta}(z')z'I' + (N - 1)f_{\delta}(z)zI$  for algebraic simplicity. This is equivalent to assuming a large population size and no local mate competition.

To determine the effect of the amount of resources available for reproduction on selection for inflorescence design, we assume that resource availability determines the total number of flowers produced,  $R$ , and that  $R$  equals the number of flowers per inflorescence unit,  $z$ , times the number of units,  $I$ . Substituting  $I = R/z$  and  $I' = R'/z'$  into equation (2) and canceling yields

$$w(z', z) = f_{\varphi}(z')R' + \frac{f_{\varphi}(z)f_{\delta}(z')R'}{f_{\delta}(z)}. \quad (3)$$

Increases in resource availability (which increase  $R$ ,  $R'$ ) increase RS through both female and male function by increasing the magnitude of both terms on the right side of the equation. This is consistent with the results of studies that show increasing RS through both gender functions with increasing total flower number (e.g., Broyles and Wyatt 1990a).

In such context dependent, sex-allocation problems, the operation and predicted outcome of selection can be explored

utilizing the game theoretical “evolutionarily stable strategies” (ESS) approach (Maynard Smith 1982). Using this technique, the fitness of an individual is calculated in a population in which the average strategy may or may not be different from that individual’s. The ESS is the strategy (reproductive, in this case) that, when dominant in the population, has equal or greater fitness than all other feasible strategies. Individual selection should move the population toward such ESSs (Maynard Smith 1982; Lloyd 1988; Charov 1989). For our problem, the ESS can be found by determining the inflorescence-unit size,  $z'$ , that maximizes individual fitness in a population with inflorescence-unit size,  $z$ , then setting  $z = z' = \hat{z}$ , the ESS inflorescence-unit size. We find the fitness-maximizing inflorescence-unit size by first differentiating the fitness function with respect to  $z'$ :

$$\frac{\delta w(z', z)}{\delta z'} = R' \left[ \frac{\delta f_{\varphi}(z')}{\delta z'} \right] + R' \left[ \frac{f_{\varphi}(z)}{f_{\delta}(z)} \frac{\delta f_{\delta}(z')}{\delta z'} \right]. \quad (4)$$

Then, setting the derivative equal to zero yields:

$$\frac{\delta f_{\varphi}(z')}{\delta z'} = - \frac{f_{\varphi}(z)}{f_{\delta}(z)} \frac{\delta f_{\delta}(z')}{\delta z'}. \quad (5)$$

Setting  $z = z' = \hat{z}$  yields

$$\frac{\delta f_{\varphi}(\hat{z})}{f_{\varphi}(\hat{z}) \delta \hat{z}} = - \frac{\delta f_{\delta}(\hat{z})}{f_{\delta}(\hat{z}) \delta \hat{z}}, \quad (6)$$

which simplifies to:

$$- \frac{\delta \ln f_{\varphi}(\hat{z})}{\delta \hat{z}} = \frac{\delta \ln f_{\delta}(\hat{z})}{\delta \hat{z}}. \quad (7)$$

Equation (7) gives the ESS condition for selection on inflorescence-unit size. Selection will favor a change in inflorescence-unit size until the proportional decrease in female RS per flower equals the proportional increase in male RS per flower (or vice versa). For example, the population is at an ESS if a one-flower increase in inflorescence-unit size results in a 10% decrease in per flower seed production, but also results in a 10% increase in per-flower pollen removal. If the population is not at an ESS, selection favors changes in inflorescence-unit size that tend to establish the equality in equation (7). This might not be possible if per flower seed production and per flower pollen removal either both increase or both decrease with inflorescence-unit size. The ESS for the former case is a single, large inflorescence unit and for the latter case is many single-flowered inflorescence units (boundary conditions).

Note that the number of flowers that can be produced for a given amount of resources,  $R'$ , does not appear in the ESS condition. Changing resource availability changes total flower number, but not the inflorescence-unit size that maximizes fitness. Rather, the ESS depends on the proportional change in per flower reproductive success through male and female function.

## METHODS

### *Study Site and Species*

We studied selection on the inflorescence design of *Asclepias tuberosa* L. (Asclepiadaceae) at the Nature Conservan-

TABLE 1. Flower visiting “taxa” of *Asclepias tuberosa* at Canelo Hills Cienega, 1992–1993, and their codes. See Fishbein and Venable (1996) for a complete species list. Insects belonging to a single family may belong to more than one visitor taxon, as noted.

“Taxon”	Composition
APIS	<i>Apis mellifera</i> (Hymenoptera: Apidae)
BATT	<i>Battus philenor</i> (Lepidoptera: Papilionidae)
BOMB	<i>Bombus sonorus</i> (Hymenoptera: Apidae)
LEPM	Medium-sized butterflies (Lepidoptera: Hesperidae, in part; Nymphalidae; in part, Pieridae, in part)
LEPS	Small-sized butterflies (Lepidoptera: Hesperidae; in part, Lycaenidae; Nymphalidae, in part; Pieridae, in part)
MBEE	Medium-sized bees (Hymenoptera: Anthophoridae, Megachilidae)
SBEE	Small-sized bees (Hymenoptera: Halictidae)

cy’s Canelo Hills Cienega Preserve, approximately 75 km southeast of Tucson, Arizona (31°33½'N, 110°31½'W, elevation 1500 m), at the lower elevational limit of Madrean oak woodland. The study site was located on the edge of a marsh, in the bottom of a shallow canyon. The study population of *A. tuberosa* was composed of 700–800 individuals in an area of approximately 0.1 ha on the lower portion of a moderate east-facing slope. Stems arising from what appeared to be the same root stock (within approximately 30 cm) were considered to belong to the same genet. Although *A. tuberosa* reproduces vegetatively from large storage roots, the roots do not appear to spread extensively. Identification of genetic individuals was rarely problematic, and flower coloration (which was diverse among individuals) often confirmed the correct identification of genets. Interplant distances among individuals varied greatly, from less than 1 m to over 10 m.

*Asclepias tuberosa* is a herbaceous, largely self-incompatible (Wyatt and Broyles 1994), perennial milkweed common throughout the eastern two-thirds of the United States, and reaching southeastern Canada and northwestern Mexico. Although commonly referred to as “Butterflyweed,” plants in this population were visited by a diverse assemblage of insects (Table 1) and were most effectively pollinated by bees (Fishbein and Venable 1996). Each plant in this population produced from one to more than 30 stems. The flowering region of each stem of an individual of *A. tuberosa* is composed of up to four branches near the stem apex. Each branch bears as many as six umbels, each of which is composed of six to 24 flowers ( $\bar{x} = 12.0$ ) (Woodson 1954; Wyatt 1980; Fishbein unpubl. data). Umbels on a branch develop sequentially towards the branch apex, such that flowers on only two (or more rarely three) adjacent umbels are open simultaneously.

Umbels on a branch are spaced rather closely, suggesting that they could potentially act as a single unit of attraction to pollinators. Similarly, flowers on different branches of a stem, stems of an individual plant, and even closely spaced plants could conceivably form a single attractive unit. Although we have no data explicitly demonstrating that umbels are the primary unit of attraction, our observations suggest that most flower visitors do not consistently end a foraging

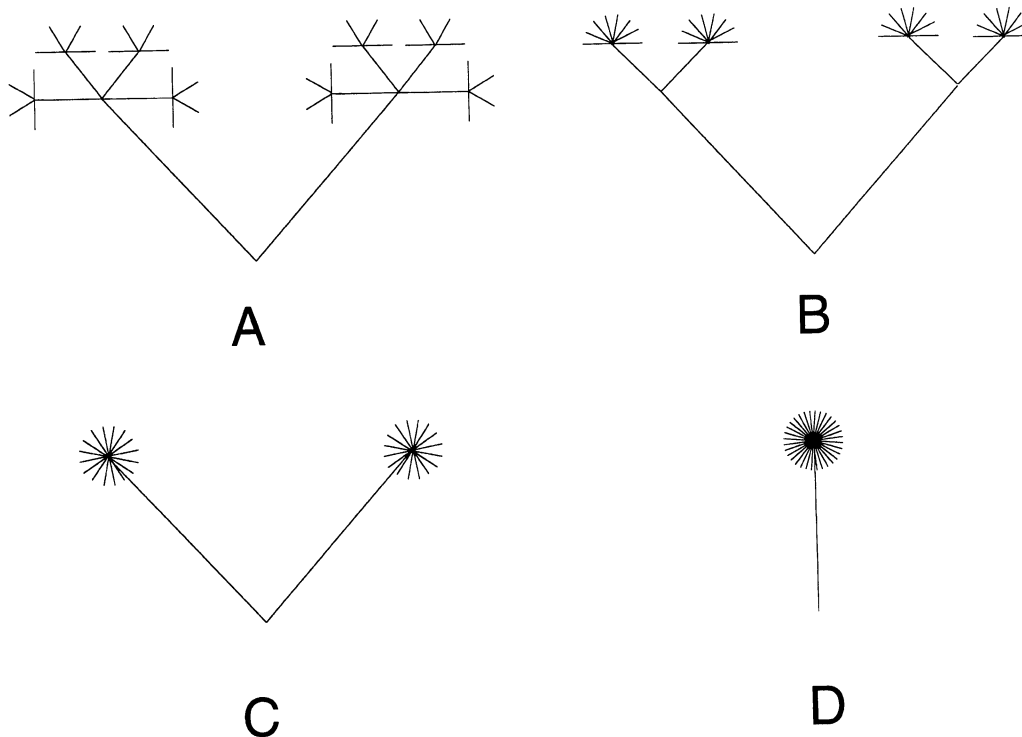


FIG. 1. Schematic representation of inflorescence-design treatments (see methods). Each small segment represents one flower. Longest segments do not represent higher orders of design structure; all designs were constructed on a single stem. The design manipulations from 1992, in which total floral display was held constant at 32 flowers, are represented here.

bout at a “patch” of flowers after visiting a single umbel, plant, or group of closely spaced plants. Where possible, we have randomized or stratified treatment applications (see data collection) so as to control for potentially confounding variation in attractive units (e.g., interplant distances, stem number). The degree to which we have been able to demonstrate differences among inflorescence designs in reproductive success is indirect evidence for the importance of the umbel as a unit of attraction.

#### Data Collection

Data were collected over two-week periods in June and July of 1992 and 1993, during the peak of flowering of *A. tuberosa* at this site. The earliest individuals began flowering about two weeks prior to the beginning of the observation periods, and the last flowers were produced about four weeks following the end of these periods. The observation periods were concluded in both years when the first sustained “monsoonal” storms occurred, after which the activity of floral visitors diminished drastically.

The inflorescence design of individual plants was manipulated by removing flowers from umbels and by tying umbels from the same stem together to simulate larger umbels. Flowers were removed by cutting the flower stalk (pedicel) near the base with a razor blade. Umbels were tied together with string around their stalks (peduncles). These modifications were made on 80 individuals in 1992 and 100 individuals in 1993. Treatments were assigned according to a stratified random scheme. Plants of five size classes (1, 2, 3, 4, 5–6 stems) were assigned randomly to treatments within each size class.

No plants with more than six stems were used because the small numbers of these plants precluded replicating treatments within plant-size class. All inflorescences on experimental plants were bagged prior to anthesis using a coarse mesh cloth that prevented transfer of pollinia (which were carried only on tarsi by flower visitors at this site; Fishbein and S. Buchmann unpubl. data), but did not exclude frequent nectar consumption by some visitors (i.e., *Bombus*, *Battus*). This type of pollination bagging has been shown to have little effect on the physical environment (e.g., temperature, humidity, airflow) of bagged flowers (Wyatt et al. 1992).

In both years of the study, four experimental treatments were applied to produce a range of designs that differed in floral density (number of flowers per unit volume), but not total flower number (Fig. 1). Experimental treatments were applied to a single stem per plant, the flowers on all other stems remaining bagged, except in 1993, when some plants had replicate treatments applied to a second stem. In 1992, the total flower number of each plant was held constant at 32 flowers. In treatment A, flowers were removed to create a display of eight umbels of four flowers each. This design was the most diffuse of the treatments and had the lowest floral density. Flowers were also removed for treatment B, which consisted of four umbels of eight flowers each. Treatment C occasionally required the removal of a few flowers to achieve a display of two umbels of 16 flowers each. For treatment D, two umbels were tied together and a few flowers were removed to create a single “umbel” of 32 flowers. Treatment D was the most compact design with the highest floral density. The experiment was repeated in 1993 with the

following modifications. Total display size was set at 24 flowers. Thus, treatments A–D were composed of 3, 6, 12, and 24 flowers per umbel, respectively. This change was implemented because treatment D (32-flowered umbels) in 1992 had low reproductive success (see results) and was outside the range of natural umbel-size variation in this population. The manner in which treatments C and D were applied was also modified in 1993. In 1992, fewer flowers were removed to create designs C and D than A and B. To control for this potentially confounding difference, in 1993 treatments C and D were applied by first removing flowers to create six-flowered umbels, then tying them together to achieve either 12- or 24-flowered umbels. Thus, treatments B, C, and D each had the same number of flowers removed, on average. Each treatment was replicated on 20 plants in 1992 and 25 plants in 1993 (distributed approximately equally among size classes), although sample sizes were reduced slightly in both years due to flower senescence or storms on sampling dates.

Female RS of each plant was measured by pollinia insertion, fruit initiation, and fruit maturation. Male RS was measured by pollinia removal. Pollinia removal and insertion were measured approximately 24 h following the removal of pollination bags from each plant. Pollination bags were removed when all bagged flowers were open. This resulted in displays composed of flowers of varying ages; however, during observation periods, flowers appeared “fresh” and functional (flowers in this population remained in this condition for up to 7 d;  $\bar{x} = 5.0$ ). Plants with any wilted flowers were eliminated from the study. Flowers on a plant (on the same stem or on nonexperimental stems) that were not part of the treatment remained bagged and were often visited by insects that could access the nectar within. However, there was no systematic difference among treatments in the number of bagged flowers. In 1992, the total numbers of pollinia removed and inserted were recorded for a single umbel (4, 8, 16, or 32 flowers for treatments A–D, respectively) on each plant. In 1993, the total numbers of pollinia removed and inserted were recorded for the entire floral display (24 flowers) on each plant. Pollinia removal was scored by noting the number of corpuscula missing from each flower (a corpusculum is the black structure that connects paired pollinia; it is readily visible in the flower prior to removal). Pollinia insertion was scored by noting the number of pollinia projecting from between the anther wings (the entrance to the stigmatic chamber where pollen germination takes place). A projecting pollinium indicates that the other connected pollinium of a pair is inserted in the stigmatic chamber. Because pollinaria observed on insect legs often have one pollinium broken off, with the corpusculum remaining on the insect (Robertson 1886; Woodson 1954; Wyatt 1976; Fishbein and Buchmann unpubl. data), it is likely that counting projecting pollinia underestimates the total number of insertions. To determine whether our estimate of pollinia-insertion rate was biased with respect to inflorescence design, we replicated the design treatments in 1993 on a second stem of each of 22 multistemmed plants chosen from among the experimental plants described above. For each replicated design, all flowers were harvested approximately 5 d following the removal of pollination bags and preserved in FAA. These flowers were

examined subsequently, and the number of projecting pollinia and total number of pollinia inserted were recorded.

The number of fruits initiated was recorded approximately 30 d following the application of the experimental treatment to each plant. Fruit initiation was defined as deflection and swelling of the pedicel and swelling of the ovary. The number of fruits matured was recorded approximately 75 d following the application of the experimental treatment to each plant. Fruits were considered mature when they reached the minimum size of fruits that dehisce naturally and release mature seeds. Unfortunately, fruit set was exceptionally low in the experimental population during the course of the study, and no mature fruits were produced on experimental plants (see results, discussion). As with pollinia-transfer data, fruit initiation was recorded on single umbels in 1992 and whole displays in 1993.

Each plant was also observed during numerous 30-min intervals in both years to determine the visitation rate (number of visits per 24 or 32 flowers per observation period) and visit duration (s) of each visitor taxon. These observations were staggered throughout the day when visitors were active and in a variety of weather conditions (Fishbein and Venable 1996). The visitor “taxon” was either a species or a broader category such as “medium-sized bee” (see Table 1). Total observation time was 105 h in 1992 and 127.5 h in 1993.

#### *Statistical Analysis*

Differences among inflorescence designs in components of male and female RS were tested with analysis of variance (ANOVA) as implemented by the GLM procedure of the SAS system under Windows (SAS Institute 1991). Significance tests are based on Type III sums of squares (SAS Institute 1989) utilizing a critical value of  $\alpha = 0.05$ . For these tests, only the seven most common visitor taxa were included (see Table 1) to avoid highly unbalanced analyses (Shaw and Mitchell-Olds 1993). In some cases the distribution of residuals from these analyses differed significantly from normal. If possible, residuals were normalized by transforming the raw data. Thus, visitation rate in 1992 was square-root transformed and mean visit duration in both years was transformed by taking the logarithm of  $1 + \text{mean visit duration}$ .

Pollinia-insertion and fruit-initiation data could not be transformed to meet the assumption of normally distributed residuals because of the large number of zeros in these data. This was also true for two-way analyses involving inflorescence design and visitor taxon. The assumptions of rank-transformed analysis of variance (Conover 1971; Potvin and Roff 1993) are also violated for these data because the large number of zeros create too many ties (Conover 1971, p. 256). For the analysis of these kinds of data, we have found randomization tests to be a useful approach (Manly 1991; Fishbein 1996; Fishbein and Venable 1996). An empirical  $F$ -distribution was created by repeatedly randomizing the association of the dependent variable with either inflorescence design (one-way analyses) or inflorescence design-taxon combination (two-way analyses) and reanalyzing the untransformed data. After 1000 random reanalyses, the actual value of  $F$  was compared to the distribution of  $F$  generated by the randomization procedure to determine the probability of ob-

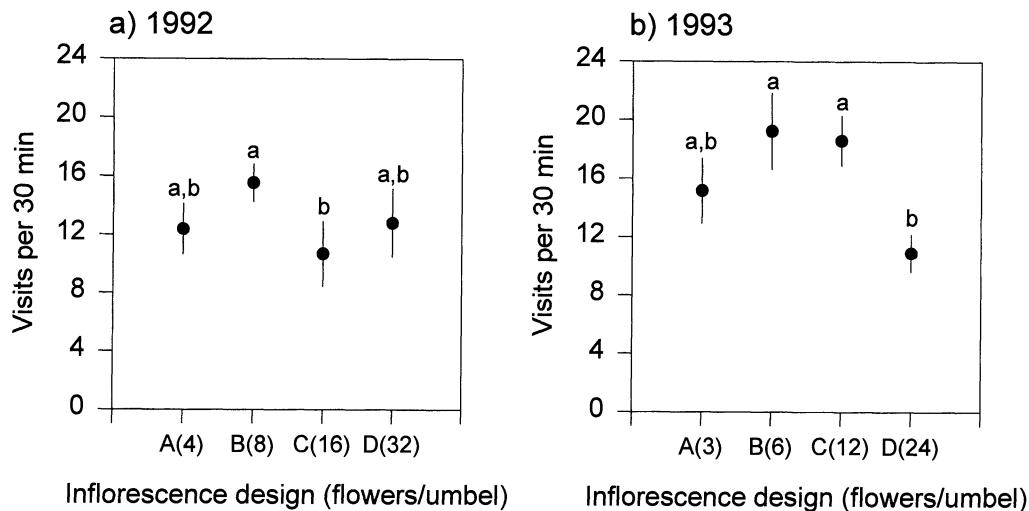


FIG. 2. Mean visitation rate  $\pm$  SE to four inflorescence designs during 30-min observation periods in (a) 1992 ( $N = 73$  plants for all designs); and (b) 1993 ( $N = 86$ ). Inflorescence designs labeled with the same letter are not significantly different (Tukey's HSD test). See methods for explanation of inflorescence-design treatments.

taining a value of  $F$  that large or larger (cf. Fishbein and Venable 1996).

In cases where inflorescence design or visitor taxon accounted for a significant proportion of the variation in the component of RS, means were compared by Tukey's Honestly Significant Difference (HSD) test (SAS Institute 1989), which controls the experimentwise Type I error rate ( $\alpha = 0.05$ ).

Because multiple visits per plant were utilized for the measurement of per visit pollinia transfer, it could be argued that those observations are pseudoreplicates (Hurlbert 1984). We compensated for the potential lack of independence of observations from the same plant by reducing the denominator degrees of freedom for the  $F$ -tests to reflect the number of plants observed rather than the number of visits recorded. This conservative approach, which may underestimate the statistical significance of our results, is analogous to the use of Box's Conservative Correction in repeated measures analyses (Milliken and Johnson 1984, pp. 359–360).

The relationship between the number of projecting pollinia per flower and the total number of insertions was examined by correlation and regression analyses (SAS Institute 1989). Analysis of covariance was used to test for biased estimates of total insertions across designs (SAS Institute 1989). In this analysis, a significant interaction between inflorescence design and the number of projecting pollinia would indicate that the relationship between projecting pollinia and total insertions varied across designs.

## RESULTS

### Visitation Rate and Visit Duration

In both years, visitation rate (per flower per 30-min observation period) was significantly higher for an intermediate inflorescence design than for one composed of larger umbels (Fig. 2; ANOVA, 1992 [visitation rate square-root transformed]:  $df = 3,69$ ,  $F = 2.51$ ,  $P = 0.066$ ; 1993:  $df = 3,82$ ,  $F = 3.81$ ,  $P = 0.013$ ). In 1992, the differences among designs

were due primarily to treatment B having a higher visitation rate per 30-min observation period than treatment C (Fig. 2a, Tukey's HSD test:  $P < 0.05$ ). In 1993, two intermediate designs (B, C) received significantly more visits per observation period than the most compact design (D) (Fig. 2b, Tukey's HSD test:  $P < 0.05$ ). An intermediate design had a higher visitation rate than the one composed of the smallest umbels in both years, however, the pairwise differences were not significant. Mean visit duration by all visitor taxa combined did not differ among inflorescence designs in either year (ANOVA; 1992:  $df = 3,63$ ,  $F = 0.59$ ,  $P = 0.62$ ; 1993:  $df = 3,82$ ,  $F = 0.72$ ,  $P = 0.55$ ).

### Pollinia Removal

Male RS, as measured by pollinia-removal rate (per flower per day), was higher in both years for a design composed of intermediate-sized umbels than one composed of larger umbels (Fig. 3; ANOVA, 1992:  $df = 3,65$ ,  $F = 2.50$ ,  $P = 0.067$ ; 1993:  $df = 3,89$ ,  $F = 5.23$ ,  $P = 0.002$ ). Similarly, in both years, pollinia removal was greater for an intermediate inflorescence design than for one composed of the smallest umbels. Pollinia-removal rate varied with inflorescence design in a pattern similar to that for visitation rate (compare Figs. 2, 3). The strength of the pattern, the statistical significance, and the correspondence with visitation rate were all stronger in 1993. In 1992, the pollinia-removal rate was greater for an intermediate design (C) than for the most compact design (D) (Fig. 3a, Tukey's HSD test:  $P < 0.05$ ). In 1993, the two intermediate designs (B, C) had greater removal rates than the most compact design (D) (Fig. 3b, Tukey's HSD test:  $P < 0.05$ ), as was found for visitation rate (Fig. 2b).

### Pollinia Insertion

In 1992, there was a marginally significant trend for female RS, as measured by pollinia-insertion rate (per flower per day), to decline with greater numbers of flowers per umbel (Fig. 4a; ANOVA [probability levels determined by random-

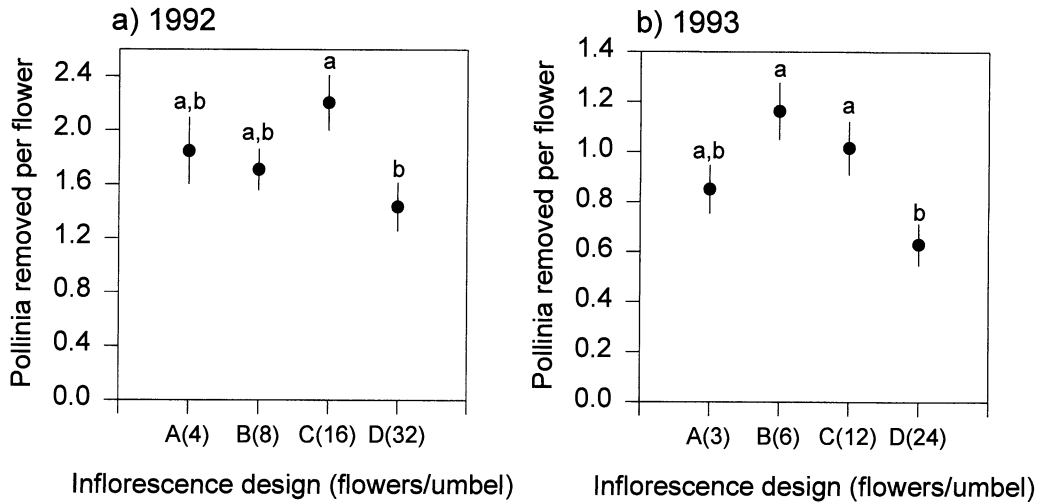


FIG. 3. Mean pollinia removed per flower  $\pm 1$  SE in 24 hr for four inflorescence designs in (a) 1992 ( $N = 69$ ); and (b) 1993 ( $N = 93$ ). Inflorescence designs labeled with the same letter are not significantly different (Tukey's HSD test). Note that vertical scales differ on the two graphs.

ization test], 1992:  $df = 3,65$ ,  $F = 2.32$ ,  $P = 0.066$ ; 1993:  $df = 3,89$ ,  $F = 1.59$ ,  $P = 0.20$ ). The greatest difference in pollinia-insertion rates in 1992 was between the inflorescence design with the fewest flowers per umbel (A) and all others; however, none of the pairwise comparisons among inflorescence designs was statistically significant (Tukey's HSD test:  $P > 0.05$ ). Differences among inflorescence designs in pollinia-insertion rate were not significant in 1993 (Fig. 4b), although the design with the most flowers per umbel (D) had the fewest pollinia inserted per flower.

The number of projecting pollinia was strongly correlated with the actual number of pollinia inserted (Pearson's  $r^2 = 0.80$ ,  $df = 20$ ,  $P < 0.0005$ ). The relationship between projecting pollinia ( $x$ ) and total insertions ( $y$ ) was linear (least-squares regression:  $y = 2.30x + 1.39$ ); residuals were normal and did not suggest a nonlinear trend, and adding a second order term to the regression equation did not account for

significantly more variance than the linear equation ( $t = 0.37$ ,  $df = 19$ ,  $P = 0.72$ ). The regression slope indicates that our measures of pollinia insertion rates are underestimated by a factor of 2.30.

Projecting pollinia are an unbiased estimate of total insertions across inflorescence designs (ANCOVA; design-by-projecting pollinia interaction:  $F = 0.31$ ,  $df = 3,14$ ,  $P = 0.82$ ; design main effect:  $F = 1.62$ ,  $df = 3,14$ ,  $P = 0.23$ ). The regression slope of projecting pollinia on total insertions did not vary across designs (nonsignificant interaction) and the y-intercept did not vary across designs (nonsignificant main effect).

*Fruit Production*

In 1992, inflorescence designs differed significantly in number of fruits initiated per flower, a second measure of

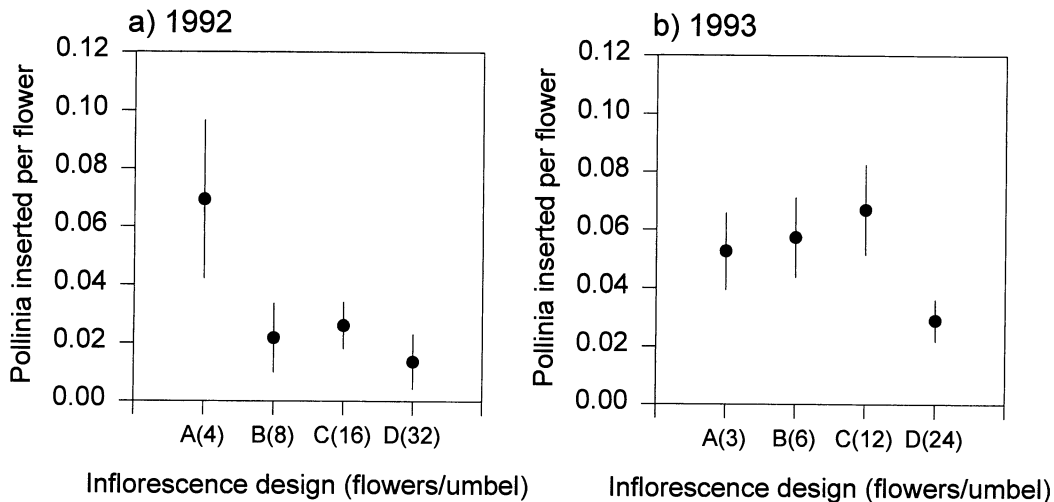


FIG. 4. Mean pollinia inserted per flower  $\pm 1$  SE in 24 h for four inflorescence designs in (a) 1992 ( $N = 69$ ); and (b) 1993 ( $N = 93$ ). Inflorescence designs are not significantly different.

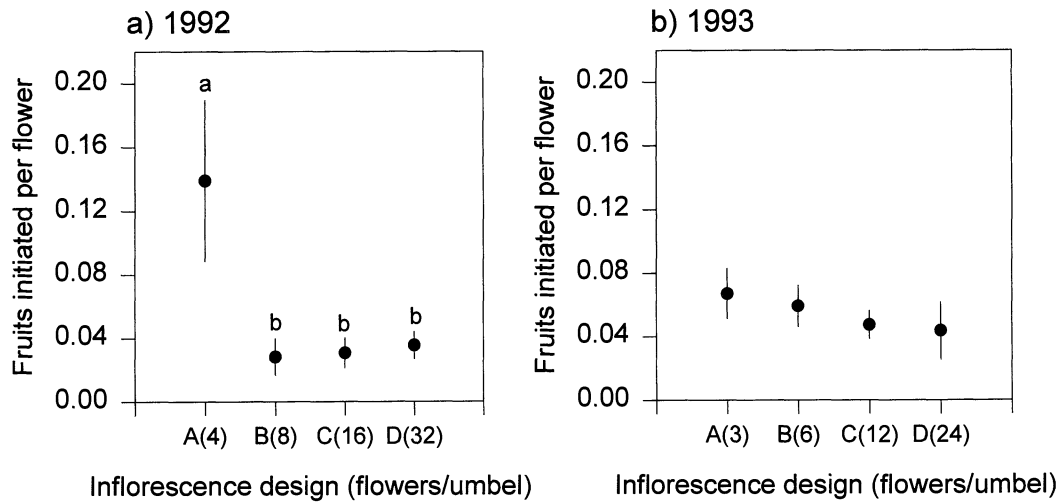


FIG. 5. Mean fruit initiation per flower  $\pm 1$  SE in 30 d for four inflorescence designs in (a) 1992 ( $N = 69$ ); and (b) 1993 ( $N = 93$ ). Inflorescence designs in 1992 labeled with the same letter are not significantly different (Tukey's HSD test).

female RS (Fig. 5a; ANOVA [probability levels determined by randomization test], 1992:  $df = 3,65$ ,  $F = 4.13$ ,  $P = 0.006$ ; 1993:  $df = 3,89$ ,  $F = 0.57$ ,  $P = 0.64$ ). The pattern of fruit initiation with inflorescence design corresponded very well with that for pollinia-insertion rate (compare Figs. 4a, 5a). The design with the fewest flowers per umbel (A) had a higher rate of fruit initiation than all other designs (Fig. 5a, Tukey's HSD test:  $P < 0.05$ ). As was the case for pollinia-insertion rate, there was no significant difference among designs in fruit-initiation rate in 1993 (Fig. 5b). There was a trend in 1993 for more compact designs to initiate fewer fruits per flower than diffuse designs.

No fruits were matured by experimental plants in either 1992 or 1993. This extraordinarily low fruit set was also exhibited by other individuals in the population that were

not part of the study. Overall fruit set in the population was estimated to be less than 0.01% in 1993 (Fishbein unpub. data).

*Variation in Visitation Rate among Pollinators*

Visitation rate during 30-min observation periods varied significantly among taxa of flower visitors in both 1992 and 1993 (Fig. 6, Table 2; Fishbein and Venable 1996). In 1992, the differences among taxa were independent of inflorescence design (Fig. 6a, Table 2). In 1993, however, differences among visitor taxa in visitation rate depended on inflorescence design (Fig. 6b, Table 2). The highest visitation rates in 1993 were by *Apis*, which visited intermediate inflorescence designs (B, C) at the highest rates and were the most

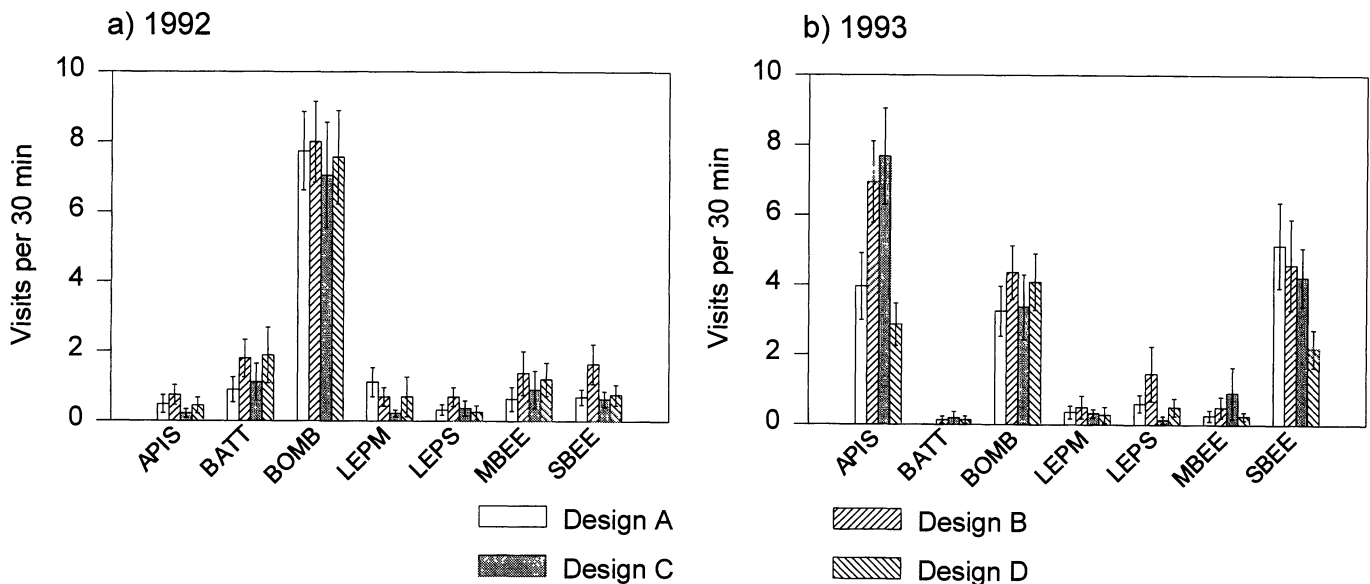


FIG. 6. Mean visitation rate  $\pm 1$  SE for the seven most common visitor "taxa" (see Table 1) to four inflorescence designs during 30-min observation periods in (a) 1992 ( $N = 210$ ); and (b) 1993 ( $N = 255$ ).



TABLE 2. ANOVAs of the effect of inflorescence design and visitor taxon on visitation rate of potential pollinators (see Fig. 6). Only the seven most common visitor taxa are included (see Table 1). Degrees of freedom were reduced to adjust for potential nonindependence of observations of visits to the same plant. Total number of visits observed was 511 (1992) and 602 (1993). Probability levels were determined by randomization test (see methods).

Year	Source	df	SS	F	P
1992:	Design	3	28	1.38	0.26
	Taxon	6	2910	71.80	0.001
	Design $\times$ taxon	18	25	0.21	0.99
	Residual	182	3260		
1993:	Design	3	125	4.12	0.005
	Taxon	6	2480	40.90	0.0001
	Design $\times$ taxon	18	382	2.10	0.003
	Residual	227	5790		

frequent visitors on them. *Apis* were much more frequent visitors overall in 1993 (Fig. 6), and their preference for intermediate designs was responsible for the higher overall visitation rate to these inflorescences (see Fig. 2b). *Bombus* visited all inflorescence designs at approximately equal rates in 1993, but were the most frequent visitors of the most compact design (D) (Fig. 6b). *Bombus* were the most frequent visitors in 1992 (Fig. 6a), and their lack of discrimination among designs may have been responsible for the weaker tendency for intermediate designs to receive the highest overall visitation rates in 1992 (see Fig. 2a).

#### DISCUSSION

Male RS per flower of *Asclepias tuberosa* (as estimated by pollinia removal per flower) was higher for intermediate inflorescence designs than for the most compact design, although this pattern was less pronounced in 1992 than in 1993 (Fig. 3). In both years, an intermediate design had the highest removal rate, although in neither year was this rate significantly higher than that for the most diffuse design (i.e., smallest umbels). Female RS (as estimated by both pollinia receipt and fruit initiation per flower) was highest for the most diffuse inflorescence design in 1992, but there was no clear trend in 1993 (Figs. 4, 5). Visitation rate, a component of both male and female RS, was highest for intermediate inflorescence designs (Fig. 2). Visitation rate corresponded closely to pollinia-removal rate in 1993, but neither visitation rate nor pollinia-removal rate corresponded to measures of female RS in either year (compare Figs. 2, 3 and Figs. 4, 5).

The few studies that have measured both visitation rates of pollinators and more direct components of male and female RS have not found a consistent relationship between visitation rate and RS. Frequent visitors of *Senna* (*Cassia*) *reticulata* (Fabaceae) were effective at both pollen removal and deposition (Snow and Roubik 1987). In *Phyla incisa* (Verbenaceae), however, visitation rate corresponded better with male than female RS (Cruzan et al. 1988), as in our study. Bell (1985) found a poor correspondence between visitation rate and both male and female RS in *Asclepias syriaca*. Broyles and Wyatt (1995) reported that large umbels of *A. exaltata* had the highest visitation rate, which corresponded better with male than female RS. However, this pattern is not ev-

ident when these quantities are measured on a per flower basis (our calculations).

Between-year differences in the relative abundance of flower-visiting taxa may have been responsible for between-year differences in the relationship between inflorescence design and male RS. *Bombus* were effective pollinators (Fishbein and Venable 1996) that had equal per flower visitation rates across designs in both years, while *Apis* were effective pollinators that visited intermediate designs with the greatest frequency (Fig. 6). When *Bombus* were the most frequent flower visitors (1992), there was a weak relationship between umbel size and both visitation rate and pollinia removal; however, when *Apis* were the most frequent visitors (1993), there was a stronger relationship between umbel size and components of male RS (Figs. 2, 3). Variation among insect taxa did not help explain the relationship between umbel size and components of female RS (compare Figs. 4, 5, 6; Fishbein 1996).

Overall, there were surprisingly few differences in per-flower RS of different designs that could be ascribed to taxonomic differences among pollinators. Initially, we predicted that the great taxonomic and morphological diversity among flower visitors would have resulted in clear differences in pollination effectiveness across inflorescence designs (cf. Schemske and Horvitz 1984; Ashman and Stanton 1991). Such differences would occur if, for example, larger visitors treated diffuse designs as a single umbel (by standing on one umbel and feeding at another), whereas smaller visitors treated each umbel as a separate unit. Individuals of all taxa, however, flew between umbels to forage at them (Fishbein pers. obs.).

There are two caveats regarding our measurements of RS in this study. First, pollinia removal is only one component of male RS. Had we been able to follow pollinia from removal to their ultimate fate, we would have been better able to estimate total fitness via male function for each design. Using pollinia-removal rate to estimate male fitness assumes a linear relationship between this component and male RS. This assumption may be violated in several ways, including differences in pollination effectiveness that depend on the composition of the flower visitor pool (Stanton et al. 1991, 1992; Thomson and Thomson 1992). Such differences may occur, for example, when the effectiveness of a pollinator taxon decreases in the presence of another, inferior visitor taxon (Thomson and Thomson 1992). Pollinia-removal rate in milkweeds, however, may be a good estimator of male RS; using paternity exclusion analysis, Broyles and Wyatt (1990a) found a strong correlation between pollinia-removal rate and realized male RS.

Second, because none of the experimental plants matured fruit over the course of the study, our measurements of components of female RS (pollinia insertion and fruit initiation) do not correspond to realized female RS. However, pollinia insertion and fruit initiation were correlated and they are likely to be strongly correlated with realized female RS in times and at places where fruits mature successfully. The causes of reproductive failure during the course of the study are not readily apparent. Pollen limitation seems unlikely, because there was substantial fruit initiation among experimental plants. Further, the total flower number of experi-

mental plants was much smaller than that of unmanipulated plants, reducing the probability of geitonogamy, which could reduce the probability of successful outcross fertilization. However, our scoring of fruit initiation may not be an accurate measure of successful *compatible* pollination. S. Broyles (pers. comm.) has observed ovary enlargement in *A. exaltata* following self-incompatible pollination. It is unknown how common this phenomenon is, or whether it occurs in *A. tuberosa*. Nonetheless, the pattern of fruit initiation for different umbel sizes (Fig. 2) does correspond well with that for fruit set measured in other milkweeds (Chaplin and Walker 1982; Broyles and Wyatt 1995). Although we have no data on plant resource status, the most plausible explanation for extremely low fruit set in this population would seem to be resource limitation, which has been documented for some milkweed populations (Chaplin and Walker 1982).

#### *The Importance of Male Function and the Evolution of "Inflorescence Size"*

The relative importance of male and female RS to the evolution of "inflorescence size" has received much attention, but with little agreement on methodology or interpretation (e.g., Willson and Rathcke 1974; Wyatt 1980; Queller 1985; Broyles and Wyatt 1990a, 1995). By focusing on the total RS of individuals that vary in size of total floral display, Wyatt, Broyles, and others concluded that selection on inflorescence size through male and female function is equally important (Lynch 1977; Wyatt 1980; Shannon and Wyatt 1986; Broyles and Wyatt 1990a). By emphasizing per flower or per umbel RS of individuals varying in umbel size, other investigators have concluded that the importance of selection on umbel size is greater through male than female function (Willson and Rathcke 1974; Chaplin and Walker 1982; Queller 1983, 1985; Bell 1985; Broyles and Wyatt 1995). The model presented in this paper suggests that it is more appropriate to consider per flower measures of male and female RS and to control for resource status (i.e., plant size or total number of flowers) rather than to compare fitness differences of plants that differ in resource status.

The relative importance of male function is intuitively compelling: Bateman's principles predict that selection on traits that affect attractiveness (e.g., flower number) should be selected more strongly through male function, if female function is resource limited (e.g., Queller 1983). Studies that have found male function to be especially important are also compelling in that umbel size typically was manipulated artificially, which potentially controlled for confounding correlates of total flower number. Such correlations might occur in some species of *Asclepias* if, for example, plants growing in unshaded sites are larger, thus producing more flowers, and receive higher visitation rates from pollinators that preferentially forage in such sites.

Results from studies of other taxa show little support for differences in the importance of selection on flower number through male and female function. Both per flower male and female RS have been found to increase with natural variation in flower number (e.g., in Orchidaceae [Schemske 1980] and Gentianaceae [Dudash 1991]). Schmid-Hempel and Speiser (1988) manipulated flower number in *Epilobium* (Onagra-

ceae) and found no difference between large and small displays in per flower RS through male or female function. In a study utilizing natural variation in flower number in *Ionopsis* (Orchidaceae), however, Montalvo and Ackerman (1987) found that male RS per flower increased with total flower number, whereas female RS per flower decreased.

Clearly, discrepancies in the results of these studies are due in part to differing concepts of "inflorescence size" (i.e., total floral display versus inflorescence-unit size). It is plausible that the relationships of male and female RS with flower number may vary with the hierarchical level of the total floral display. In particular, changes in female RS with flower number per inflorescence unit may be quite different than those with total flower number, if each inflorescence unit obtains resources for fruit production from a separate resource pool (cf. Schoen and Dubuc 1990). Most importantly, variation in total flower number due to varying resource status has not been addressed in these studies. Broyles and Wyatt (1990a, 1995) have emphasized that plant-level reproductive success is more strongly correlated with total floral display (e.g., stems/plant, umbels/plant, flowers/plant) than with the size of inflorescence units (i.e., flowers/umbel). However, total flower number responds plastically to plant resource status in modular organisms (Harper 1977): individuals with greater access to resources are likely to be larger, to produce more flowers (through the production of more inflorescence units), and have higher male and female RS (e.g., Eckhart 1991). Broyles and Wyatt (1990a) have reported that variation in umbel size is as great within plants as between them. We suggest that this variation is due primarily to developmental factors (e.g., acropetal production of smaller umbels in *Asclepias*; Willson and Rathcke 1974; Chaplin and Walker 1982; Fishbein pers. obs.), although we have not studied this variation in detail. Umbel size (which varies at most by a factor of 3–4; Broyles and Wyatt 1990a; Fishbein unpubl. data) is a more constant feature of the floral display than total display size, which can vary by two orders of magnitude (Fishbein unpubl. data), and is more likely to represent a fixed *genetic* component of inflorescence design. Selection may operate on umbel size even if there is developmental variation within individuals. Variation in resource status obscures evolutionary trade-offs between the size and number of plant structures, which are key to understanding selection on the size of these structures (e.g., Venable 1992).

#### *The Evolution of Inflorescence Design*

The confounding effects of variation in resource status can be removed theoretically or experimentally by distinguishing fitness due to varying design decisions (e.g., inflorescence unit size versus number) and that due to the effect of varying resource availability. This approach has been successful in modeling the size of offspring (Smith and Fretwell 1974; Lloyd 1987; Venable 1992) and similar approaches have been applied to modeling allocation to structures that differentially serve male and female function (Lloyd 1984, 1988; Charlesworth and Charlesworth 1987). Our model explicitly considers the roles that male and female function play in the evolution of inflorescence design. Our principal result is that

selection favors a balance between proportional changes in female and male RS with changes in inflorescence-unit size.

Schoen and Dubuc (1990) emphasized the role of fixed costs of inflorescence units on the evolution of inflorescence design. They modeled male and female fitness functions as fixed attributes of the size of inflorescence units, independent of the frequency of inflorescence designs in a population. They demonstrated that for various combinations of shapes of fitness functions and sizes of fixed costs, there exist ESSs for small, intermediate, and large units. These ESSs occur when the proportional advantages of inflorescence-unit size and number are balanced (Lloyd 1987; Schoen and Dubuc 1990). Our model assumes a linear fitness function for number of inflorescence units and does not consider fixed costs of inflorescence production (for a relaxation of these assumptions, see Venable in press). However, it incorporates frequency dependence in the male and female fitness functions for inflorescence-unit size.

#### *Selection on Inflorescence Design in A. tuberosa*

To apply our general theoretical result to empirical data for *A. tuberosa*, a more explicit model that incorporates details of the reproductive biology of *Asclepias* can be developed (Fishbein 1996). In such a model, the rate of pollinia removal per flower is the appropriate measure of male function and the rate of fruit production per flower is the appropriate measure of female function. Both of these rates are functions of umbel size. Analysis of such a model (Fishbein 1996) results in a special case of the more general model presented in this paper: changes in umbel size are favored until a proportional change in per flower pollinia-removal rate is balanced by an equal but opposite proportional change in fruit production per flower. For any special case, the equality given in equation (7) will be expressed in terms of the male and female fitness components that vary on a per flower basis with inflorescence-unit size. In the case of *Asclepias*, these are likely to be pollinia removal per flower and fruit set per flower.

When our empirical results for the consequences of varying umbel size on male and female RS are applied to the model, the predicted ESS umbel size is very close to the population and species mean for *A. tuberosa*. For female function, above the smallest umbel size (1992), or for all umbel sizes (1993), per flower RS is approximately constant (Figs. 4,5). Thus,  $\delta \ln f_{\delta}(z)/\delta z$  equals zero at all but sometimes the smallest umbel sizes. Accordingly, equation (7) predicts that the ESS will be found where  $\delta \ln f_{\delta}(z)/\delta z$  equals zero, which will be at the maximum per flower pollinia-removal rate (Fig. 3). This peak occurs at an intermediate umbel size in both years (although it is not statistically distinguishable from the smallest umbel size), roughly at the mean of 12 flowers per umbel found for this population (Fishbein unpubl. data) and for the species across its range (Wyatt 1980; Fishbein unpubl. data). For umbel sizes larger than 16 flowers, selection through male function would tend to favor smaller umbels, whereas that through female function would be indifferent to umbel size. For umbel sizes smaller than six flowers, selection through male function appears to favor larger umbels (though the difference is not statistically significant), whereas that

through female function would be indifferent (1993) or might favor smaller umbels (1992 data). Our data are not precise enough to determine which gender function would impose the strongest selection on small umbel sizes at times when the female fitness function is like that found in 1992. If the apparent peak in male function at intermediate sizes is not real, our data would suggest that smaller umbel sizes (i.e., < 12 flowers) are selectively neutral.

The match of the predicted ESS umbel size for *A. tuberosa* with observed umbel sizes is based on two years of data at a single location; these conditions may not be representative of those under which umbel size has been selected. In particular, our strongest pattern for a peak in male function occurred in a year when nonnative *Apis* were the most common pollinators. In southeastern Arizona, *Apis* are abundant at most low- to mid-elevation sites, and have been for some time (S. Buchmann pers. comm.). Although *Apis* did not originally select for ancestral umbel sizes in *A. tuberosa*, they may behave in a manner similar to the pollinators that did and they are certainly important present-day selective agents. Despite these caveats, we find it provocative that observed umbel sizes match the predicted ESS.

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