THE ROLE OF MALE FLOWERS IN ANDROMONOECIOUS SPECIES: ENERGETIC COSTS AND SIRING SUCCESS IN *SOLANUM CAROLINENSE* L.

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Two non-mutually exclusive hypotheses regarding the benefits of andromonoecy (producing perfect and female-sterile flowers on the same plant) are tested using *Solanum carolinense.* Results indicate that (1) staminate flowers are cheaper to produce than perfect flowers, even after correcting for their relative position in the inflorescence; (2) the resources saved by producing staminate flowers are not re-allocated to other fitness enhancing functions; and (3) the main morphological characteristic of staminate flowers, pistil reduction, does not increase either pollinator visitation or siring success of open-pollinated plants. These results indicate that neither the *resource savings hypothesis* nor the *increased pollen donation hypothesis* explains the evolution and maintenance of andromonoecy in *S. carolinense*.

KEY WORDS: Andromonoecy, flower size, pollen donation, resource allocation, siring success, *Solanum carolinense*.

One of the main foci of plant evolutionary biology over the past quarter century has been to understand the processes responsible for the tremendous variety of mating systems exhibited by plants (Barrett 2002a). One mating system that is peculiar to plants is andromonoecy, in which individuals produce both perfect and staminate (i.e., female-sterile) flowers. Andromonoecy occurs in approximately 4000 species (approximately 2%) of flowering plants (Yampolsky and Yampolsky 1922; Richards 1986; Miller and Diggle 2003).

Two, not necessarily mutually exclusive, hypotheses have been proposed regarding the source of selection that favors the evolution of female-sterile flowers. The *resource reallocation hypothesis* posits that the production of staminate flowers reduces resource investment in functionally male flowers and permits the resources saved to be re-allocated toward other fitness-enhancing traits (Ruiz Zapata and Kalin Arroyo 1978; Primack and Lloyd 1980; Bertin 1982; Solomon 1985; Spalik 1991; Emms 1993). Support for this hypothesis is provided by the common observation that a variety of traits in staminate flowers are smaller than in perfect flowers (Smith 1931; Primack and Lloyd 1980; Dulberger et al. 1981; Solomon 1986; Anderson and Symon 1989; Diggle 1991a; Emms 1993; Podolsky 1993; Spalik and Woodell 1994; Manicacci and Despres 2001; Huang 2003; Cuevas and Polito 2004; Diggle and Miller 2004), although in most cases these comparisons have not corrected for the tendency for male and perfect flowers to be produced in different locations and/or at different times (see below). Moreover, few attempts have been made to determine whether resources saved are redirected to other fitness-enhancing traits (but see Elle 1998, 1999).

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1

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The *increased pollen donation hypothesis*, by contrast, posits that staminate flowers are more effective at donating pollen than perfect flowers for any of several reasons: staminate flowers may produce more or larger pollen; staminate flowers may experience reduced pollen–pistil interference, either within flowers or among flowers on the same plant; or staminate flowers may be more attractive to pollinators (Solomon 1985; Podolsky 1992; 1993; Harder and Barrett 1996; Elle and Meagher 2000; Barrett 2002b). Evidence supporting this hypothesis is equivocal. Depending on the species examined, pollen production/viability of staminate flowers has been observed to be greater (Huang et al. 2000; Huang 2003), equal to (Solomon 1985, 1986; Manicacci and Despres 2001; Cuevas and Polito 2004), or less than (Spalik and Woodell 1994) that of perfect flowers. And although reduced pollen–pistil interference has been suggested as the mechanism favoring staminate flowers in some species (e.g., *Solanum carolinense*, Elle and Meagher 2000; Connolly and Anderson 2003), direct evidence for this type of sexual interference is scarce in andromonoecious taxa (Podolsky 1992, 1993).

We report here results from an investigation designed to determine whether either of these hypotheses provides a compelling explanation for the evolution of andromonecy in *Solanum carolinense* L. (Solanaceae). We address the resource reallocation hypothesis by asking (1) whether allocation to staminate flowers is reduced and (2) whether plants with a higher proportion of staminate flowers exhibit increases in other fitness components. We evaluate the increased pollen donation hypothesis by determining whether staminate flowers are more attractive to pollinators and whether reduction in pistil size increases the effectiveness of pollen donation.

Material and Methods

STUDY SPECIES

Horsenettle, *Solanum carolinense* L. (subgenus Leptostemonum, Solanaceae), is a self-incompatible perennial herb native to the southeastern United States. It reproduces both sexually (seeds), and clonally through spreading roots. Individual plants produce both staminate (male) and perfect (hermaphroditic) flowers. Perfect flowers are usually borne at the base of the inflorescence and staminate near the top (Solomon 1985). Staminate flowers in *S. carolinense* have reduced styles that do not extend beyond the anthers, reduced ovaries, and are incapable of setting fruit even when they are artificially pollinated (Solomon 1985). *S. carolinense* produces pollen as the only reward to pollinators and is buzz-pollinated by large bees. Individual fruits have an average of 160 seeds (Elle 1999).

TESTING THE RESOURCE REALLOCATION HYPOTHESIS

A complete test of the resource reallocation hypothesis requires determining (1) whether resources are saved by producing staminate flowers; and (2) whether resource savings translates into an increase in one or more fitness components. We examined whether resource savings occur by asking whether staminate flowers are smaller than perfect flowers *at the same position on an inflorescence*. Although previous reports on this species indicate that, on average, staminate flowers are smaller (Solomon 1986), these studies do not account for the fact that gender and inflorescence position are confounded. It is thus possible that staminate flowers are smaller than perfect flowers simply because they tend to be produced on the distal end inflorescences where even perfect flowers are generally smaller (Diggle and Miller 2004). We asked whether saved resources translate into increases in other fitness components by asking whether there is a trade-off, manifested by a genetic correlation (Charlesworth and Morgan 1991), between staminate flower production and various fitness components.

Resource savings

To assess resource savings, we compared various measures of the sizes of perfect and staminate flowers corrected for inflorescence position. A total of 150 plants (consisting of clonally propagating 10 ramets from each of 15 genets collected from an abandoned field near Duke Forest, Durham, NC) were grown in a greenhouse at Duke University. Soon after flowering began, flowers were collected thrice-weekly from all individuals. To stimulate the production of staminate flowers (Diggle 1991b) a randomly chosen 25% of perfect flowers were pollinated during each census. This pollination level results in a similar level of fruit set as that observed in natural populations (Wise and Cummins 2002). All other flowers were collected and fixed in a 3:1 ethanol:acetic acid solution for subsequent measurements. During collection, the rank position from the base of the inflorescence was recorded for each flower.

Sizes of preserved flowers were measured using a digital caliper and a Leica MZ6 dissecting microscope equipped with a ruled objective. Eight different measurements were taken from each flower: (1) petal length, (2) petal width, (3) anther length, (4) anther width, (5) style length, (6) stigma width, (7) ovary length, and (8) ovary width. Measurements 1–4 were taken from a single petal or anther chosen at random. In addition, a subset of 45 randomly chosen flowers of each morph were subsequently used to determine pollen number and size using the protocols described by Solomon (1986). Four replicate measurements were made for each flower in a hemacytometer, and the mean was used in statistical analyses.

To distinguish statistically between positional and gender effects on floral size, we fitted each of the eight size characters using linear mixed-effects models (LME) with gender and flower rank as well as flower rank^2 and flower rank^2 * gender as fixed effects, and inflorescence rank, ramet, and genet as random effects using the routine "PROC MIXED" (SAS 2005). Significance of

fixed effects was estimated using Type III sum-of-squares. Model selection for fixed effects was done through stepwise deletion of non-significant quadratic (flower rank^2) and higher order interactions (flower rank^2 $\times$ gender) according to the marginality principle (Zar 1974; Fox 1997).

Resource reallocation

To determine whether resources saved in the production of staminate flowers were allocated to other fitness-enhancing functions, we estimated the genetic correlations between, on one hand, the proportion of flowers that were staminate (PSF), and on the other hand, flower production, and seed production in the field. Such genetic correlations are commonly used to assess allocation trade-offs (e.g., Mazer and Delesalle 1998; Charlesworth and Morgan 1991; Ågren and Schemske 1995). Similar correlations were estimated previously for PSF and above-ground biomass, survival, and ramet production by Elle (1999).

To generate experimental plants, 136 fruits were collected haphazardly (one fruit per plant) from an abandoned field near Duke Forest, Durham, North Carolina. Six seeds per fruit were germinated and grown in 1.68 L pots containing Farfard 3P soil mix (Farfard, Agwam, MA) arranged in six spatial blocks under a 14 h daylight regime. Plants from the greenhouse were crossed in a North Carolina II design (Lynch and Walsh 1998), to produce 30 paternal half sib families, each with contributions from 10 female parents (270 full-sib families total). In the summer of 2003, seeds from these crosses were germinated in the greenhouse and, after approximately 5 weeks, transplanted to two experimental field populations in a field containing native *S. carolinense*. Two seedlings from each of 80 full-sib families chosen to encompass the 30 paternal half-sib families were randomly assigned to each of two spatial blocks within each population, for a total of 320 seeds per population.

Plants were censused during the summer of 2004. Because of clonal reproduction in the previous year, more than one ramet per plant was present in 2004. Of these, we allowed only one ramet to flower and on alternate days recorded the number and gender of all flowers produced. Fruits were collected after the first frost of the year and maximum diameter (d) of each fruit was measured to estimate the number of seeds produced (s) using the relationship $s = 70.1 - 23.0d + 2.18d^2 - 0.0415d^3$ ($n = 114$ fruits). Wise (2003) demonstrated that this relationship explains 90% of the variation in seed number in *S. carolinense*. Genetic correlations were estimated by calculating both full- and half-sib family means (Falconer and MacKay 1996).

TESTING THE INCREASED POLLEN DONATION HYPOTHESIS

The primary question we addressed in this experiment was whether pistil reduction in staminate flowers affects male success. One approach to addressing this issue would be simply to compare the male success of naturally produced staminate and perfect flowers. However, an experiment of this type would necessarily confound the effects of flower gender with the effects of flower position and size (Diggle 2003; this study), because perfect flowers tend to be produced lower on an inflorescence and, as a result, tend to be larger. To avoid this confounding, we instead chose to "create" staminate flowers from perfect ones by removing the style and stigma. We then assessed the relative siring success of these plants and plants with perfect flowers using arrays of potted plants in the field.

Plants for experimental arrays were generated from nine genets from the eastern United States that had specific *S*-locus genotypes, which served as markers for paternity analysis. Each genet was clonally replicated to produce between six and 12 ramets per genet, which were then grown in 1.68 L pots in Farfard 4P soil mix in the greenhouse. This set of plants formed the base pool from which we drew to establish experimental trials. Each experimental trial consisted of exposing an array of plants to pollinators in a garden outside the Duke Biology Greenhouses for a single day. Although the average life-span of individual flowers in the field is about 2 days (unpubl. results), experimental flowers exposed to pollinators for a single day were heavily visited and achieved very high fruit set. Each array consisted of 22 plants arranged in a polygonal grid and exposed to natural pollinators for one day, after which they were returned to the greenhouse to allow seeds to mature. No naturally occurring flowering *S. carolinense* were observed within 500 m.

Each array consisted of eight pollen recipients with between one and six unmanipulated flowers, seven pollen donors with three perfect flowers each, and seven pollen donors with three artificially created "staminate" flowers each. We chose to use staminate-only plants, rather than plants that had both staminate and perfect flowers, to minimize pollen–pistil interference among flowers in staminate plants, and thus maximize our ability to detect siring differences among staminate and perfect flowers.

To create a staminate flower, the pistil was removed at its base from the original perfect flower. In addition, to avoid biases in pollinators' behavior due to touching the flowers, flowers in the perfect treatment were also manipulated by the experimenter but the pistil was left intact. Plants of the three categories were placed in alternating positions within the grid.

To assess the relative male success of perfect and staminate flowers, we used different *S*-locus genotypes for the two types of flowers on any given day. Which ramets were used was dictated by availability of open flowers. On average each experimental trial included 5.16 genets allocated among the three treatments. Genotypes for recipients were chosen to be fully compatible with both types of donors. To control for effects of genetic background, trials were run in pairs. In each pair, the same genotypes were

used for pollen recipients, with the genotypes of the perfect and staminate plants switched between trials.

To elucidate paternity, seeds were extracted from the fruits produced by recipient plants and a random sample of two to four seeds per fruit were germinated. Each recipient plant produced on average 1.76 fruits per trial (14 fruits on average per trial, 169 fruits total). Genomic DNA was extracted from leaf tissue using a modified CTAB protocol (Varadarajan and Prakash 1991) and used as template for separate PCR reactions using allele-specific primers for each possible paternal allele. In total 504 seeds were genotyped. These primers were developed by Y.-Q Lu and are listed in the Appendix (Table A1). Products were scored on 1% agarose gels stained in ethidium bromide. In a few cases more than one paternal allele was amplified, and the DNA sample was considered to be contaminated and that offspring excluded from the analysis.

Relative siring success of perfect and staminate flowers was compared using a standard likelihood approach (e.g., Ritland 1990) in which we assumed that the probability that a particular seed was sired by pollen from a staminate plant was ρ. Under the null hypothesis of equal siring success, the expected value of ρ is 0.5 because the arrays contained equal numbers of perfect and staminate flowers. The significance of deviation from this expectation was evaluated by determining whether the support for the observed value of ρ was more than two log-likelihood units greater than support for the expected value (Edwards 1992; see e.g., Fry and Rausher 1997).

To determine whether there are differences in pollinator visitation rates to the two floral morphs, we conducted pollinator observations in our experimental arrays on six days. Observation periods of 15 min were conducted between 6:15 and 9:00, after which visitation rates dropped markedly due to high temperatures and low pollen availability. For each pollinator entering the array we recorded its identity, the number and gender of flowers and plants visited, the duration of each floral visit, and the sequence of visitation, until it left the array. We considered true visits to be only visits lasting 1 sec or longer (shorter visits are highly unlikely to result in pollen removal in this buzz-pollinated species, M. Vallejo-Marín pers. obs.).

Because preliminary analyses indicated that the proportion of visits to staminate plants in our array experiment did not differ among trials (likelihood ratio test, $\chi^2 = 4.68$, df = 5, $P = 0.456$), visits were pooled across all trials for subsequent analyses. Under the null hypothesis of no pollinator preference for staminate plants, visits to staminate plants should constitute half of all visits to donor plants. Deviation from this expectation was tested using a standard likelihood approach (Zar 1974) assuming that the probability of visiting a staminate plant can be modeled as a binomial probability (e.g., Jones 1997).

Number of flowers visited per plant and duration of visits per flower were compared among treatments using analysis of variance with flower gender as the main effect. All analyses were carried out using the statistical package R version 2.1.1.

Results
TEST FOR RESOURCE SAVINGS

Here we ask whether producing staminate flowers saves resources by comparing the sizes of staminate and perfect flowers. To deconfound the effects of flower size and flower position along the inflorescence, we asked whether staminate and perfect flowers *at the same position* differ by including floral rank as a covariate and asking whether the gender or gender × rank effects were significant.

In general, all eight morphological characters except pollen number and size decrease in size with increasing floral rank (Fig. 1A–J). In some cases (e.g., petal length, anther length, ovary length and width, and style length), this relationship is curvilinear, as indicated by the significant rank^2 effect (Fig. 1), whereas in other characters the relationship appears linear. More importantly, the best-fitting linear or quadratic regression of character on floral rank for perfect flowers lies above, or at the same level as, the corresponding regression for staminate flowers, indicating that for a given floral rank, the character is larger, or at least no smaller, in perfect flowers. These differences are statistically significant ($P < 0.022$) for all characters except petal width, as indicated by the significant gender effect in the analysis of covariance (Fig. 1). By contrast, neither pollen number nor pollen size is detectably influenced by floral rank (Fig. 1I, J: nonsignificant rank effect), suggesting strong selection for developmental canalization in these characters. Moreover, gender has no detectable effect on pollen number. After correcting for floral rank, pollen is still slightly larger for perfect flowers, but the difference is small (approximately 2%).

Overall, these results indicate that *at the same position along an inflorescence*, staminate flowers are smaller than perfect flowers. There is thus a resource savings associated with substituting a staminate flower for a perfect flower.

TEST FOR RESOURCE REALLOCATION

If resources saved by producing smaller staminate flowers are reallocated to other fitness-enhancing functions, there should be a positive genetic correlation between PSF and fitness components. In the field experiment, however, full-sib and paternal half-sib family mean correlations between PSF and flower and seed production were all negative, five of them significantly so even after Bonferroni corrections (Table 1). Moreover, using just the paternal half-sib family mean correlations and pooling over
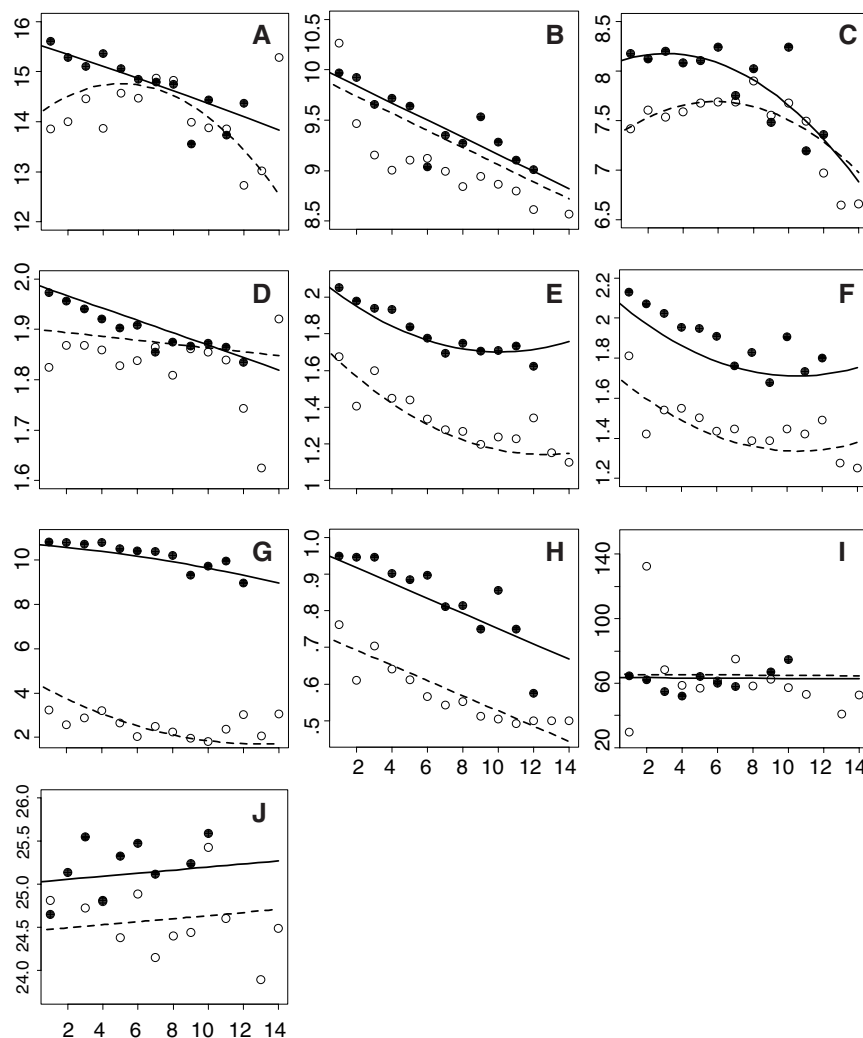
Figure 1. Relationship between flower position (rank, x-axis) and floral size characters. Flower rank is assigned according to the position of the flower along each inflorescence, starting with the most basal flower. Solid and open circles represent perfect (hermaphroditic) and staminate (male) flowers, respectively. Lines represent the regression estimated in the analysis of variance. (A) petal length; (B) petal width; (C) anther length; (D) anther width; (E) ovary length; (F) ovary width; (G) style length; (H) stigma width; (I) pollen number; (J) pollen diameter; units of measurement as follows: (A–H) mm; (I) counts ($\times$ 10^4); (J) μm. Significant effects in the models are: (A) rank**, rank $\times$ gender*, $rank^2$*, $rank^2$ $\times$ gender*; (B) rank****; (C) gender*, rank*, rank $\times$ gender*, $rank^2$***; (D) gender***, rank***, rank $\times$ gender*; (E) gender****, rank****, rank $\times$ gender*, $rank^2$***; (F) gender****, rank****, $rank^2$****; (G) gender****, rank****, rank $\times$ gender****, $rank^2$ $\times$ gender**; (H) gender****, rank****; (I) NS; (J) gender*. Significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Table 1. Full- and half-sib family mean correlations in field conditions between proportion of staminate flowers (PSF) and flower and seed number. The correlations are shown for transformed data (arcsine-square root PSF; natural log flower number; natural log [seed number + 1]). Nominal statistical significance (*P*-value) was calculated using a two-sided *t*-test with the null hypothesis that the true correlation does not differ from zero. Sample sizes are shown in parentheses. Significant correlations, after Bonferroni corrections, are in bold.

Character	Full-sibs	*P*-value	Paternal half-sibs	*P*-value
Flower number (population 1)	−0.052 (65)	0.673	**−0.534 (24)**	**0.004**
Flower number (population 2)	**−0.371 (78)**	**0.004**	−0.247 (28)	0.187
Total seed number (poulation 1)	**−0.353 (65)**	**0.003**	**−0.722 (24)**	**<0.001**
Total seed number (population 2)	**−0.387 (78)**	**<0.001**	−0.074 (28)	0.697

populations using a combined probability test (Sokal and Rohlf 1995), the negative correlations between PSF and flower number and between PSF and seed number are both significant ($\chi^2 = 14.4$ and 14.5 respectively, df $= 4$, $P < 0.01$ in both cases). Our results thus provide no evidence of resource reallocation.

TEST FOR EFFECTS OF PISTIL REDUCTION ON POLLINATOR VISITATION

Because the primary morphological difference between staminate and perfect flowers is pistil size, we examined the effect of pistil absence on pollinator visitation and male success. The only pollinators that visited our experimental arrays were bumblebees, *Bombus pennsylvanicus* and *B. impatiens*, with the former accounting for approximately 87% of all visits. We recorded a total of 166 pollinator bouts in 5.3 h of observations, during which we observed 1000 flower visits.

Our observations provide no evidence that pollinators preferentially visit plants with staminate flowers: the proportion of visits to donors that were visits to staminate plants was 0.509 (95% CI $= 0.472$–0.545), which is not significantly different from the expected 0.5 (deviance $= 0.240$, df $= 1$, $P = 0.624$).

The number of flowers visited per plant also did not differ detectably between staminate and hermaphroditic plants. Pollinators probed the same number of flowers in both male and hermaphroditic individuals (2.09 ± 0.08 and 2.09 ± 0.09 visits respectively, $F_{1,288} = 0.01$; $P = 0.896$). Likewise, the average time (in seconds) that pollinators spent on each flower (staminate $= 4.39 \pm 0.27$, perfect $= 4.10 \pm 0.18$) or on each plant (male $= 9.34 \pm 0.66$, hermaphrodite $= 9.39 \pm 0.83$) did not differ between the two morphs ($F_{1,288} = 0.23$, $P = 0.6317$; and $F_{1,288} = 0.16$, $P = 0.683$, respectively). These results provide no evidence that staminate flowers are more attractive to pollinators.

TEST FOR EFFECTS OF PISTIL REDUCTION ON SIRING SUCCESS

Using experimental arrays we tested the increased pollen donation hypothesis by assessing whether plants with staminate flowers are more successful pollen donors than plants with perfect flowers. Because the probability of siring success by male plants (ρ) did not differ among days (deviance $= 6.372$, df $= 6$, $P = 0.382$) or among replicate trials (deviance $= 2.736$, df $= 5$, $P = 0.740$), we compared siring success of the two treatments by pooling data across all days. The overall siring success of staminate plants was indistinguishable from their frequency among the pollen donor group (expected $\rho = 0.5$, observed $\rho = 0.510$, 95% CI $= 0.446$–0.575, deviance $= 0.109$, df $= 1$, $P = 0.741$). Consequently, we infer that staminate morphology does not confer an increased male success over perfect morphology in similar-sized flowers.

Discussion

RESOURCE-REALLOCATION HYPOTHESIS

One of the hypotheses for the evolution and maintenance of andromonoecy that we have examined in this investigation is that production of staminate flowers is advantageous because it allows reallocation of resources from gynoecial structures, and perhaps other flower parts, to other fitness-enhancing characters (Ruiz Zapata and Kalin Arroyo 1978; Primack and Lloyd 1980; Bertin 1982; Solomon 1985; Spalik 1991; Emms 1993). Two types of evidence are required for support of this hypothesis: (1) it must be demonstrated that producing staminate flowers instead of perfect flowers saves resources and (2) it must be demonstrated that this savings is actually redirected.

In *Solanum carolinense*, it is clear that fewer resources are invested in an average staminate flower than in an average perfect flower. It has been shown previously that perfect flowers are on average larger than staminate flowers in this (Solomon 1986; Elle 1998) and other species (Diggle 1991b) of *Solanum*. However, it is also well known that in *Solanum* and other andromonoecious plant species, flower size typically decreases along an inflorescence, whereas at the same time perfect flowers tend to occur lower, and staminate flowers higher, on an inflorescence (Diggle 2003; Miller and Diggle 2003). This pattern means that an average difference in size between staminate and perfect flowers could be due simply to their different positions on an inflorescence. However, in the context of the evolution of andromonoecy, it is critical to determine whether staminate flowers are smaller than perfect flowers *at the same position*, because the evolutionary transition from hermaphroditism to andromonoecy presumably involves converting some perfect flowers to staminate flowers.

Our analyses provide strong support for such a difference in *S. carolinense*. For seven of eight anatomical characters measured staminate flowers are smaller than perfect flowers at the same position. Because nitrogen content is similar for the two genders (Solomon 1986), the smaller size means that less nitrogen as well as less energy is invested in staminate flowers. Furthermore, a comparison of floral longevity in the field (Vallejo-Marín and Rausher, unpublished data) indicates that staminate flowers are shorter lived than perfect flowers (1.9 ± 0.024, $n = 806$; and 2.1 ± 0.0139, $n = 2374$; mean longevity in days $\pm$ standard error, for staminate and perfect flowers respectively; n represents the number of flowers analyzed). It thus appears that genotypes that produce a greater proportion of staminate flowers have the potential to reallocate resources to other functions.

Reallocation could occur within individual flowers (e.g., increased pollen production, increased corolla size), within inflorescences (e.g., increased flower or fruit production), or within the whole plant (e.g., increased survival, increased vegetative biomass). Our results convincingly rule out reallocation within

individual flowers because staminate flowers appear to be smaller, or the same size, as perfect flowers in all characters measured. There is also no evidence that resources saved by producing staminate flowers are reallocated to inflorescence or whole-plant traits. As indicated by the genetic correlations we obtained, genotypes that produce a higher proportion of staminate flowers actually produce fewer flowers and seeds. Similarly, in a greenhouse experiment, we found a negative though non-significant, full-sib family mean correlation between PSF and root biomass ($r = -0.130$, df $=$ 85, $P = 0.226$). These results complement those of Elle (1999), who failed to find any association between PSF and above-ground plant size, survival, or ramet production.

In summary, our experiments have uniformly failed to obtain evidence indicating reallocation of resources saved by producing staminate flowers, either within flowers, within inflorescences, or within the whole plant. The lack of evidence for resource reallocation should be taken with caution for at least three reasons. First, it is possible that even our analysis using genetic correlations may fail to detect the signature of resource reallocation (i.e., positive genetic correlation between PSF and a fitness related trait), if genetic variation in resource acquisition is considerably higher than genetic variation in resource allocation, in which case correlations may appear to be of the opposite sign or absent (e.g., Van Noordwijk and de Jong 1986). Second, reallocation may have involved characters that have not been examined (e.g., defense characters). Third, it is possible that the magnitude of reallocation is too small to be detected in the experiments that have been performed. Nevertheless, based on current evidence, we conclude that resource saving and reallocation is not a major advantage of producing staminate flowers in *S. carolinense*.

INCREASED POLLEN DONATION HYPOTHESIS

A second hypothesis that we have examined is that an advantage of producing staminate flowers is that they are more successful at transmitting pollen than are perfect flowers (Bertin 1982; Podolsky 1993). Enhanced success could be achieved by a greater efficiency of pollen transfer to departing pollinators, increased pollen production, or increased pollinator visitation. The results of our array experiment provided little support for this hypothesis: perfect flowers sired just as many seeds per flower as did staminate flowers. Consistent with this outcome, pollinator visitation to the two morphs was indistinguishable in frequency or duration. Finally, our results (see Fig. 1) demonstrate that there is no detectable difference in pollen number between morphs, and pollen is actually smaller for staminate flowers, which would be expected to reduce the relative siring success of staminate flowers (e.g., Cruzan 1990).

One limitation of our experiment is that we used artificially created staminate flowers rather than natural flowers. We adopted this approach to avoid confounding flower gender with size, as would be unavoidable if we used true staminate flowers. Nevertheless, our manipulations may not have captured differences between the morphs that are relevant to pollen export. We believe this possibility to be unlikely, however, for two reasons. First, as was true in our experiment, true staminate and perfect flowers do not differ in pollen production, and this equality was maintained in our experiment. Second, true staminate flowers are smaller in most features than perfect flowers at the same inflorescence position. Because in general pollinators tend to prefer larger flowers (e.g., Bell 1985; Galen and Newport 1987; Stanton and Preston 1988; Conner and Rush 1996), we would expect true staminate flowers to experience fewer, rather than more, visits, compared to our manipulated staminate flowers. In any case, at the very least we can conclude that pistil reduction, the major morphological change associated with staminate flowers, does not enhance pollen transmission.

Our results contrast with those of Elle and Meagher (2000), who found that plants with a higher proportion of staminate flowers had a higher male fitness, even when differences among plants in flower number and flower size were controlled for statistically. Several explanations for these contrasting results are possible. One is that the relative male success of the two morphs may be context dependent. For example, it is possible that in Elle and Meagher's investigation, unlike ours, pollinators exhibited a preference for staminate flowers. A second possibility is that their attempt to control statistically for confounding effects of flower size and flower number on male success was not entirely successful. For example, their analysis only included the linear terms involving the covariates. If the true relationship is non-linear, the apparent relationship between PSF and male fitness could reflect this unaccounted-for nonlinearity. Third, in Elle and Meagher's experiments, PSF was confounded with background genotype. If, for example, plants that are genetically less vigorous (genetically "unhealthy") tend to produce a low PSF, the apparent greater male success of high PSF plants could have been due to higher vigor rather than to high PSF per se. We note that we were able to avoid this problem in our experiment by using the same genotypes reciprocally for the two flower types. Finally, it is possible that our experimental manipulation of flower gender omitted a crucial difference that occurs in the natural genders, although we believe this unlikely (see above). Until further experimentation distinguishes among these possibilities, our inference that the increased pollen donation hypothesis does not explain the evolution of staminate flowers in *S. carolinense* must remain tentative.

CONCLUSION: RELATIVE IMPORTANCE OF RESOURCE ALLOCATION AND SIRING SUCCESS FOR THE EVOLUTION OF ANDROMONOECY

Our results, coupled with those of previous workers (Solomon 1986; Elle 1998, 1999), provide little support for either the

resource-allocation hypothesis or the increased pollen donation hypothesis as explanations for the evolution and maintenance of staminate flowers in *Solanum carolinense*. This suggests that the correct explanation may lie in a third hypothesis, the increased pollen receipt hypothesis, which predicts a greater seed production by plants that produce some staminate flowers that do not interfere with the deposition of pollen in other fruiting flowers in the same plant (Podolsky 1992). In a subsequent report (Vallejo-Marín and Rausher, in press) we provide evidence indicating that this hypothesis may indeed be the correct explanation.

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Appendix

Table A1. Primer sequences used to amplify each allele at the S-locus and size of the expected product

S-allele*	Primer	Primer sequence (5′ to 3′)	Expected product size (bp)
A	A for	CAGCACGCAATGTTGAATGAC	142
	A rev	ACACAAAGTTTCTGGCGTTATG	
C	C for	CTCCTTCATCATGTATGACTG	139
	C rev	CCAATGATCAGCCTTTCTGG	
D	D for	CAAATGCTGAATGACTGCTCT	117
	D rev	CTATGGCAGGTGACACTGAA	
E	E for	AGGGTACACTGCTGCAGGA	134
	E rev	CCTTGGCGAAACAACCTTCA	
G	G for	AGTTGCGCAGACATCTACAAT	144
	G rev	CGTTCAACCTCTATGGAGAG	
H	H for	CAGATATAAAGGGCACAGTGC	146
	H rev	TCGGAAAAATCAAGGTTTCTGG	
K	K for	TAATACTGCATGACTGCCTCA	154
	K rev	CACTGGAGGTATGAGTTCAC	

*S-locus nomenclature following Richman et al. (1995).