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ECOLOGICAL AND GENETIC FACTORS CONTRIBUTING TO THE LOW FREQUENCY OF MALE STERILITY IN *CHAMAECRISTA FASCICULATA* (FABACEAE)[1]

HOLLY L. WILLIAMS[2,3] AND CHARLES B. FENSTER

Department of Plant Biology, University of Maryland, College Park, Maryland 20742-5815

Bumble bee pollinated *Chamaecrista fasciculata* provides pollen as the sole reward to its pollinators. Male sterility, expressed as an absence or nearly complete absence of pollen production, occurs in low frequency in populations of *C. fasciculata*. Here we describe experiments, using *C. fasciculata*, to examine frequently cited determinants of the spread and maintenance of male sterility: compensation and the genetic basis of male sterility. In addition, we examine the role the pollination system plays in determining the reproductive success of the male steriles. Seventeen populations in Maryland, Illinois, and Kansas were surveyed and found to range from 0 to 6% male sterility per population. An artificial population of male-sterile simulants and hermaphrodites was created to examine how the local frequency of nonrewarding male steriles might affect male-sterile female reproductive success. Male steriles performed equally poorly, with respect to seed production, whether surrounded by other male-sterile simulants or hermaphrodites. Compensation was examined by comparison of male steriles and hermaphrodites with respect to several reproductive and nonreproductive characters. Male steriles outperformed hermaphrodites in terms of nonreproductive biomass, but performed equally in terms of ovule number and produced many fewer flowers. The genetic basis of male sterility was examined by performing both intra- and interpopulational crosses of male steriles to hermaphrodites and indicate that male sterility is not purely cytoplasmic. The low frequency of male sterility in *C. fasciculata* populations may reflect reduced female reproductive success because of pollinator avoidance, lack of reproductive compensation, and a mode of inheritance that is not purely cytoplasmic.

Key words: *Chamaecrista*; compensation; dioecy; Fabaceae; frequency-dependent selection; gynodioecy; male sterility; pollinator behavior.

Male sterility in plant species has been widely documented in natural populations, and its persistence and spread have been theoretically demonstrated to be the probable first step in the evolution of dioecy (Lewis, 1941; Ross, 1970, 1978; Ross and Shaw, 1971; Lloyd, 1975, 1976; Ross and Weir, 1976; Charlesworth and Charlesworth, 1978). Given that male sterility entails the initial loss of fitness through male function, empirical investigations are needed in order to quantify the factors responsible for its introduction, spread, and maintenance, which will clarify our general understanding of the initial steps of the evolution of dioecy.

Darwin (1877) first recognized that compensation, the consequent diversion of resources from lost function to existing functions (i.e., pollen production to seed production in male steriles), may be one mechanism that allows the spread of loss of either male or female reproductive function. Compensation in terms of increased seed production of male steriles compared to conspecific hermaphroditic or monoecious individuals has been examined in a number of taxa, e.g., *Fuchsia thymifolia* and *Fuchsia microphylla* (Arroyo and Raven, 1975), *Cucurbita foetidissima* (Kohn, 1989), *Thymus vulgaris* (Atlan et al., 1992), *Scheidea globosa* (Sakai and Weller, 1991), *Phacelia linearis* (Eckhart, 1992a, b), *Trifolium hirtum* (Molina-Freaner and Jain, 1992a), and *Scheidea adamantis* (Sakai et al., 1997). Of the aforementioned, only *T. hirtum* lacked resource trade-offs.

Compensation in terms of increased seed dispersal ability (Bawa, 1980; Givnish, 1980) and avoidance of predation through saturation have also been suggested (Bawa, 1980). In addition to increased female reproductive function, the avoidance of inbreeding, or enforced outcrossing resulting in progeny of higher vigor, has been frequently cited and empirically documented as a factor for the spread of male sterility within populations (Jain, 1961; Ross and Shaw, 1971; Lloyd, 1975; Ross and Weir, 1976; Charlesworth and Charlesworth, 1978; Ross, 1978; Bawa, 1980; Kohn, 1988, 1989; Couvet et al., 1990; Sakai and Weller, 1991; Sakai et al., 1997).

The selective advantage in female reproductive success that male steriles must demonstrate is dependent on the mode of inheritance of male sterility. If male sterility is caused solely by nuclear genes, then male steriles must produce twice as much seed as hermaphrodites or their progeny must be twice as likely to survive to reproductive age in order to persist in the population (Lewis, 1941; Lloyd, 1975; Charlesworth and Charlesworth, 1978). This is because male steriles lose half their reproductive potential when they lose the ability to produce pollen. In contrast, if male sterility is caused solely by

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[2,3] Author for correspondence, current address: Science Department, Valencia Community College, Osceola Campus, 1800 Denn John Lane, Kissimmee, FL 34744.

cytoplasmic genes (maternal inheritance), then male steriles need only make slightly more seed than hermaphrodites to spread in the population (Lloyd, 1975, 1976; Charlesworth and Charlesworth, 1978; Frank, 1989), since natural selection will then act strictly upon cytotypes. If nuclear restorer alleles (alleles responsible for a complete restoration of male function) are present in a population with cytoplasmic male sterility, the requirements for persistence and spread of male sterility are complex and depend upon negative pleiotropic effects of the restorer alleles (Frank, 1989).

The above-mentioned theoretical models provide explicit predictions as to the equilibrium frequency of male steriles, which depends on the outcrossing rate of hermaphrodites, the amount of inbreeding depression expressed upon selfing, the amount of compensation expressed in females in terms of increased seed production, and the mode of inheritance of male sterility. In addition, theoretical models also predict a frequency dependency; as the frequency of male steriles rises then the fitness of hermaphrodites increases as well, since the average hermaphrodite fertilizes more ovules with its pollen (Lloyd, 1975; Ross, 1977; Charlesworth and Charlesworth, 1978). However, we know little of the ecological factors that may affect frequency-dependent selection and it seems likely that the type of pollinator or mode of reward provided to the pollinator might in part determine the equilibrium frequency of male steriles in a population. For example, if pollinators are attracted to flowers for pollen reward, then the frequency of male sterility may not be driven by the fitness of hermaphrodites, but rather by the female reproductive success of the male steriles. A priori we expect male steriles might fare well if low in a population because of ''mistake pollination'' (Baker, 1976; Dafni, 1984; Barrett, 1987), but may suffer at higher frequency if pollinators learn to avoid the male steriles.

Anecdotal observations revealed that male sterility exists in low (<5%) frequency in populations of the annual legume *Chamaecrista fasciculata* (Fabaceae). Here we examine the causes of the low frequency of male sterility by documenting the frequency of male sterility, analyzing the genetic basis of male sterility and quantifying the presence of compensation and frequency-dependent selection on male-sterile female reproductive success in *C. fasciculata*. *Chamaecrista fasciculata* offers only a pollen reward and is pollinated almost exclusively by bumble bees using buzz pollination (Thorp and Estes, 1975; Fenster, 1991a; Wolfe and Estes, 1992). The male steriles are morphologically identical to the hermaphrodites and are, therefore, Bakerian mimics of the hermaphrodites (Barrett, 1987). Consequently this system provides a model for determining the dynamics of male sterility where females (male steriles) mimic hermaphrodites in order to attract pollinators.

MATERIALS AND METHODS

Study organism—*Chamaecrista fasciculata* Michx., partridge pea, is a self-compatible, annual legume of old fields, disturbed prairie, and savanna found throughout the eastern United States (Irwin and Barneby, 1982). Flowers open at dawn, wither by dusk, and are highly outcrossing (Fenster, 1991a). Male steriles of this species can be defined as having <10 pollen grains per flower. Male steriles otherwise appear unaltered, although often they lack purple pigmentation in their anthers. Flowers on a plant are either all male sterile or all fully functional. Flowering begins in mid- to late July and continues until first frost. From one to six flowers per inflorescence are produced in axillary racemes and typically floral displays consist of 1–10 flowers open per day in the field. Flowers contain from four to 20 ovules and 10 000–35 000 pollen grains when pollen is present (Fenster, unpublished data). We have never observed values intermediate to our definitions of hermaphrodite and male-sterile flowers.

Frequency of male sterility—To quantify the frequency of male sterility in natural populations of *C. fasciculata,* seeds were taken, at random, from each of four populations in Kansas and Maryland and from seven populations in Illinois. The seeds were sown in the greenhouse in fall 1992 and flowered in early spring 1993, yielding from two to 31 adult progeny for each population ($\bar{X}$ = 15/population, SD = 10.5) and totaling 209 individuals, each representing different maternal families. Given the expectation of 5% male sterility our sample sizes had a 54% chance to detect at least one male sterile per population ($1–0.95^{15}$), and a probability >0.999 of detecting at least one male sterile overall. Upon flowering male sterility was assessed by visual inspection (forcefully tapping the stamens to determine the presence of pollen), which was verified by anther dissection of male steriles and examination at 100×. The two methods showed almost complete concordance. Two additional Maryland populations (of ~500 each) were assessed in the field for male sterility, in the early morning prior to bee visitation. A transect was made across each of the populations. Approximately every 0.5 m along the transect a plant was selected and several flowers were inspected for the presence of pollen with a total of 50 plants inspected in each population. Sex determination in this and following experiments was based on a minimum of three flowers per plant.

Genetic basis of male sterility—A cross of a male sterile to a hermaphrodite pollen parent should yield all male-sterile progeny, if male sterility is determined by cytoplasmically inherited genes (CMS). Detection of nuclear male sterility (NMS) relies upon recovery of both male steriles and hermaphrodites from crosses between male steriles and hermaphrodites or selfing of hermaphrodites. To detect the presence of nuclear-cytoplasmic inheritance (NCMS), both intrapopulational and interpopulational crosses of male steriles to hermaphrodites have been employed. This method, previously used by Sakai and Weller (1991) and Molina-Freaner and Jain (1992b), rests on the assumptions that nuclear restorer alleles will be specific to male-sterile cytotypes and that the cytotypes and/or the restorer alleles will be specific to their respective populations. Hence, if NCMS is present then the frequency of male steriles should be greater in interpopulation crosses than in the intrapopulation crosses.

All of the following crosses and growth of progeny were conducted in the University of Maryland at College Park greenhouse. The genetic basis of male sterility in *C. fasciculata* was examined using three male steriles from Gooselake Prairie (GLP) in Morris County, Illinois, which were detected in an unrelated crossing experiment in the summer of 1992. Each of these were crossed to approximately ten hermaphrodites from each of three populations: GLP, Zander Woods (Z), a population ~100 km northeast of GLP in Will County, Illinois, and Smithsonian (MD), in the Smithsonian Edgewater facility in Anne Arundel County, Maryland, ~1200 km east of GLP. Thus, a single male-sterile mother was crossed both to hermaphrodites from the same population and to hermaphrodites from two distant populations. Additionally, one GLP hermaphrodite was selfed. The male steriles were of two sterility types: two females with very little pollen ([<10 pollen grains per flower (sterility type = A)] and one female with no pollen grains per flower (sterility type = B). The progeny (F_1) were sown in late fall 1992 in the greenhouse, and plants began flowering in early spring when the presence of pollen was assessed (N = 101). The F_1 hermaphrodite progeny were selfed, retaining the cytotype of the original mother and allowing for greater examination of CMS and NCMS. This also increased the

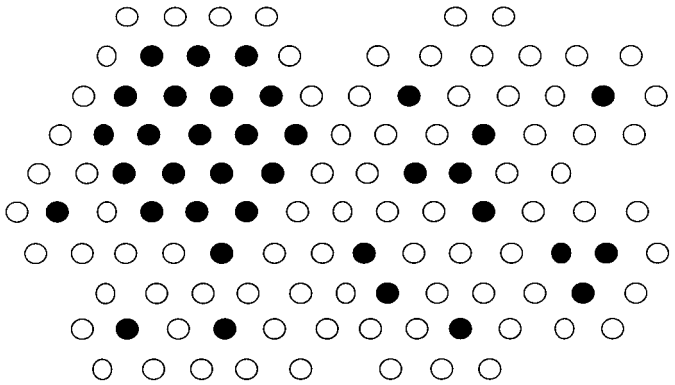

Fig. 1. Male sterile and hermaphroditic positions in the frequency-dependent selection array using *Chamaecrista fasciculata*. From day to day plants alternately occupied male-sterile positions and hermaphroditic positions. Experiment was run for 6 d and plants never occupied the same position twice. Filled circles = male-sterile positions; unfilled circles = hermaphroditic positions.

sample size for examination of NCMS. The F_2 progeny ($N = 148$) were then sown in early spring 1994 and assessed for pollen production upon flowering in early summer.

The presence of hermaphrodites in the progeny of a cross between a hermaphrodite and a male sterile was taken as evidence against CMS and a role for nuclear-inherited genes. To determine the role of NCMS vs. NMS, chi-square analysis was conducted to determine whether the frequency of male-sterile progeny was lower in intrapopulation crosses (within GLP) vs. interpopulation crosses (GLP × Z or GLP × MD).

Compensation—The F_2 mentioned above (148 individuals) were used in evaluating male-sterile compensatory effects. All of the plants, derived from the two male-sterile grandmothers with very limited pollen production, were assessed for pollen production and 26 were found to be male sterile. Plants were maintained in the University of Maryland greenhouse in a randomized design.

Data were gathered on the average number of ovules per ovary, the number of flowers produced per day, and the total nonreproductive biomass for both the hermaphrodites and the male steriles. Ovule number was quantified for three random flowers per plant for 21 male steriles and 85 hermaphrodites. Ovule number per ovary was assessed by soaking individual ovaries in bleach for 8 h and counting the number of ovules under 10× magnification. The number of flowers per day was assessed by counting the total number of flowers on each of the 148 plants twice a week over 3.5 wk (spanning peak flowering phenology) for a total of seven separate counts or measurements. Nonreproductive biomass was determined by the dry mass of the entire vegetative plant body (excluding fruits and flowers) above the cotyledonary node for all 148 plants. To control for maternal effects, male steriles and hermaphrodites derived from the same F_1 mother were compared using paired, two-tailed *t* tests ($N = 9$, 11, and 11 F_1 maternal families used to quantify compensation in terms of ovule number per ovary, estimated total flower production, and biomass, respectively).

Frequency-dependent selection—To examine whether a correlation exists between the frequency of male steriles in a population and their reproductive success and to determine whether male sterility in a pollen rewarding system adversely affects female seed production, 115 adults were grown from bulk seeds collected at GLP. The adults were placed in an experimental garden array (located on private property in Annapolis, MD) with designated male-sterile positions and hermaphrodite positions (Fig. 1). This array acted as an artificial population in which the frequency of male steriles varied from groups of six adjacent male-sterile neighbors to isolated male-sterile individuals. The design was a hexagonal mosaic pattern of equidistant individuals, which maximized the number of adjacent male-sterile plants using the smallest total number of experimental plants. We manipulated hermaphrodites to mimic the phenotype of male sterility because genetically determined male steriles may experience compensation (i.e., may produce more flowers, seeds, etc.), and it would be difficult to separate frequency-dependent selection effects from compensation effects or other factors.

Since the stamens of *C. fasciculata* release pollen via terminal pores, placing glue on the stamen tips of hermaphrodites prevents pollen dispersal and makes the plant effectively male sterile. Superglue was chosen because it dries quickly and without apparent discoloration or odor. Preliminary but unquantified observations indicated no aversion to the glue on the part of the pollinators (bumble bees), and seed set was not hampered by the presence of the glue when treated flowers were hand pollinated (glue was placed on the stamens of three flowers on each of ten greenhouse-grown plants, the flowers were hand-pollinated, and all 30 flowers set fruit). Each day of the experiment (spanning 25 July to 31 July 1994 but skipping 28 July because of inclement weather) 35 plants (those in MS positions, Fig. 1) had their stamen tips glued, and 36 plants (those in hermaphrodite positions, excluding perimeter plants) did not have their stamens altered. Hence, each day, some individuals in the array were effectively male sterile while the remainder were hermaphroditic. To control for any possible aversion to the superglue by the pollinators a droplet of superglue (approximately equivalent to the amount of superglue needed to glue the stamens on the male-sterile simulants) was placed on the petal nearest the stamens of each flower of all the hermaphrodites. As a result, bumble bees approaching any of the experimental flowers in the array encountered superglue, and the possible effects of any aversion were uniform throughout the experimental array.

To control for inherent differences in reproductive ability (production of flowers, fruit, seed, etc.), plants in designated male-sterile and hermaphroditic positions were rotated each evening during the experiment to randomly chosen positions. Plants never occupied the same position twice, and they alternated between male-sterile and hermaphrodite assignments. Thus, each individual spent a total of 3 d as a hermaphrodite and 3 d as a male sterile (alternating daily between the two) and occupied a total of six different positions. To control for any possible edge effects the perimeter plants were treated as all other hermaphrodites in the array except that they were not moved each day and were not included in the analysis. Mosquito netting was placed over the array each night and removed the next morning ~2 h after dawn when alterations to the flowers were complete. This prevented pollination before the treatments could be completed. Starting on the second day of the experiment all plants were allowed a maximum of six flowers and additional flowers were removed. This controlled for several very robust individuals observed in the array on the first day. Because subsequent analysis (below) indicated no effect of day, all days are included in the analyses.

Data were collected on the number of fruits initiated and the number of fruits matured per day and number of seeds per matured fruit. Fruit-to-flower ratios and seed-to-fruit ratios were then calculated. Fruit-to-flower ratios of male-sterile individuals were regressed on the number of male-sterile neighbors (0–6) using linear regression analysis (Statview SE+). This determined whether male steriles in positions where their nearest neighbors were male steriles show reduced fecundity when compared to male steriles whose nearest neighbors were hermaphrodites. Separate linear regression analyses were also conducted for each day of the experiment to determine whether frequency-dependent selection may have differed across days. When plants lacked flowers on a particular day the data from that day for that plant were eliminated from analysis. Untransformed data were plotted because they met the requirements of linear regression analysis. Note, experimental male-sterile simulant frequency was much larger than any female frequency observed in natural *C. fasciculata* populations. Thus, any frequency-

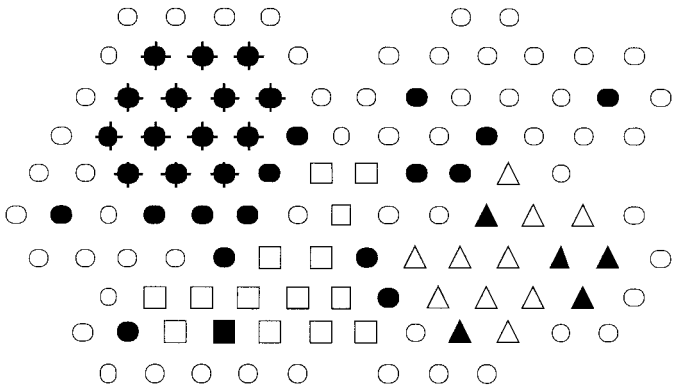

Fig. 2. Regions of high, moderate, and low male sterility in the frequency-dependent selection array using *Chamaecrista fasciculata*. Censuses were made on the frequency of bumble bee visitation to each particular region of the array for 4 d. Filled circles = male-sterile positions not included in bee visitation sample; unfilled circles = hermaphroditic positions not included in bee visitation sample; filled, hatched circles = male steriles in the region of high male sterility; filled and unfilled triangles = male-sterile and hermaphroditic positions, respectively, in the region of moderate male sterility; filled and unfilled squares = male-sterile and hermaphroditic positions, respectively, in the region of low male sterility.

dependent reduction in female reproductive success likely represents an upper limit of any natural effect.

The total average fruit-to-flower ratio per day for all hermaphrodites was compared to that of male-sterile simulants using a paired *t* test. Data were square-root arcsine transformed because of frequent low values exhibited by the male steriles. This did not improve analyses so *t* tests for unequal variances were performed (SAS, 1985). Analysis of seed per matured fruit was accomplished by performing a paired *t* test using Statview SE+.

In order to determine whether bumble bees discriminate among male-sterile simulants and hermaphrodites data were taken on the frequency of visitation to three areas of the array (Fig. 2) beginning on the third day of the experiment. The three areas were graded from high percentage of male-sterile simulants (100%) to medium (33%) and to low (6.7%) percentage of male-sterile simulants, respectively. A visit was defined as a bumble bee alighting on a plant in the given area over a 15-sec census period. Four censuses were conducted each day at 15-min intervals over the span of 1 h. A Kruskal-Wallis test was used because the residual variances were unequal and data were not normally distributed.

RESULTS

Frequency of male sterility—The survey of the greenhouse-grown plants showed only one partial male sterile (very little pollen) from Kansas. The two populations surveyed in the field in Maryland had male-sterility frequencies of 2 and 6%.

Genetic basis of male sterility—The results of crosses are shown in Tables 1 and 2. None of the crosses (F_1 and F_2) yielded 100% male sterility. There were no significant differences between intrapopulational crosses and interpopulational crosses in the F_1 ($\chi^2 = 1.2$, 1 df, $0.20 < P < 0.30$ for crosses in GLP vs. crosses to Z hermaphrodites and $\chi^2 = 0.15$, 1 df, $0.50 < P < 0.70$ for crosses in GLP vs. crosses to MD hermaphrodites). To compensate for limited sample sizes, which lead to small expected cell numbers, we also pooled data from both interpopulation crosses and found no significant difference between intra- and interpopulation crosses (frequency of male steriles = 9.7 and 15.7% for within GLP vs. pooled interpopulation crosses, respectively; $\chi^2 = 0.40$, 1 df, $0.30 < P < 0.40$). Selfed progeny from one GLP hermaphrodite had a high frequency of male-sterile progeny (33%).

The overall frequency of male steriles in the F_2 was 21.3%. The F_2 showed no significant difference between intrapopulational crosses and interpopulational crosses ($\chi^2 = 2.9$, 1 df, $0.05 < P < 0.10$ for crosses in GLP vs. crosses to Z hermaphrodites and $\chi^2 = 1.4$, 1 df, $0.20 < P < 0.30$ for crosses in GLP vs. crosses to MD hermaphrodites). Again, to compensate for limited sample sizes, we also pooled data from both interpopulation crosses and found no significant difference between intra- and interpopulation crosses (frequency of male steriles =

Table 1. Analysis of F_1 of crosses involving GLP male steriles to GLP, Z, and MD hermaphrodites of *Chamaecrista fasciculata*.

Cross	*N*	Herm.	MS	%MS
GLP × GLP (within pop.)				
GLP (A) (female 1) × GLP	8	7	1	13
GLP (A) (female 2) × GLP	14	12	2	14
GLP (B) (female 3) × GLP	9	9	0	0
Total	31	28	3	9.7
GLP × Z (100 km)				
GLP (A) (female 1) × Z	8	8	0	0
GLP (A) (female 2) × Z	23	17	6	26
Total	31	25	6	19
GLP × MD (1200 km)				
GLP (A) (female 1) × MD	15	14	1	6.7
GLP (A) (female 2) × MD	24	20	4	17
Total	39	34	5	13
GLP herm. selfed	12	8	4	33

Note: A = male steriles with very little pollen per flower; B = male steriles with no pollen; N = number of progeny; Herm. = number of hermaphrodites in progeny; MS = number of male steriles in progeny; %MS = percentage of male steriles in the F_1.

Table 2. Segregation of male sterility in the F_2 of crosses between male sterile and hermaphroditic parents of *Chamaecrista fasciculata*.

Cross	*N*	Herm.	MS	%MS
GLP × GLP selfed				
GLP (A) (female 2) × GLP	44	36	8	18
GLP (B) (female 3) × GLP	41	38	3	7.3
Total	85	74	11	13
GLP × Z selfed				
GLP (A) (female 1) × Z	13	13	0	0
GLP (A) (female 2) × Z	13	6	7	54
Total	26	19	7	27
GLP × MD selfed				
GLP (A) (female 1) × MD	28	22	6	21
GLP (A) (female 2) × MD	9	7	2	22
Total	37	29	8	22

Note: A = male steriles with very little pollen per flower; B = male steriles with no pollen; N = number of progeny; Herm. = number of hermaphrodites in progeny; MS = number of male steriles in progeny; %MS = percentage of male steriles in the F_2.

13 and 24% for within GLP vs. pooled interpopulation crosses, respectively; $\chi^2 = 2.68$, 1 df, $0.20 < P < 0.30$). A comparison between the frequency of male sterility in the F_1 and the F_2 between generations also showed no significant difference ($\chi^2 = 0.60$, 1 df, $0.30 < P < 0.50$).

Note that seed did not germinate from the selfing of hermaphrodites originally generated in the cross of GLP(A), female 1, to GLP hermaphrodites and did not germinate from the original parental crosses involving GLP(B) and hermaphrodites from Zander and MD. To reiterate, the GLP(B) female was of a different sterility type than the other two male steriles in that it did not produce any pollen. If we confine comparisons of the intra- and interpopulation crosses using females of the same male sterility type (A = producing little pollen), then the differences between the two types of crosses were even less in the F_1 (14 vs. 16%, intra- vs. interpopulation pooled, respectively) and F_2 (18 vs. 24%, intra- vs. interpopulation pooled, respectively).

Compensation experiment—On average, hermaphrodites produced more than three times as many flowers as male steriles ($X = 6.8$ and 2.0 for hermaphrodites and male steriles, respectively; $t_{[10]} = 5.07$, $P = 0.0005$). The average number of ovules per ovary for hermaphrodites vs. male steriles showed no significant difference ($X = 7.6$ and 8.2 for hermaphrodites and male steriles, respectively; $t_{[8]} = -1.41$, $P = 0.196$), while biomass of hermaphrodites was significantly less than male steriles ($X = 13.6$ and 18.0 g for hermaphrodites and male steriles, respectively; $t_{[10]} = -2.32$, $P = 0.042$).

Frequency-dependent selection experiment—Linear regression analysis demonstrated no frequency-dependent effect between individual days of the experiment and, thus, we present only the results for all days pooled together. Fruit initiation, fruit maturation, and seed set per matured fruit did not significantly increase or decrease as the number of adjacent male-sterile neighbors changed (slopes for linear regression analysis: $b = -0.006$, $t_{[171]} = 0.829$, $P = 0.409$; $b = -0.005$, $t_{[171]} = 1.01$, $P = 0.315$; and $b = 0.218$, $t_{[28]} = 0.953$, $P = 0.349$ for fruit initiation, fruit maturity, and seed per matured fruit, respectively). Polynomial regression did not significantly increase the r^2.

Hermaphrodites initiated fourfold more fruit than male-sterile simulants ($X = 0.389$ and 0.100 for hermaphrodites and male-sterile simulants, respectively; $t_{[106.8]} = -7.84$, $P = 0.0001$). Hermaphrodites also matured significantly more fruit per flower than male-sterile simulants ($X = 0.176$ and 0.024 for hermaphrodites and male-sterile simulants, respectively; paired, $t_{[76.8]} = 7.79$, $P = 0.0001$), but there was no difference in seed set per matured fruit ($t_{[17]} = 0.088$, $P = 0.931$).

Total number of individual bees during the censuses were three, 15, and 12 bees in the high-, medium-, and low-frequency male-sterile areas, respectively. These represented significant differences in bee visitation between the areas of high male sterility and moderate male sterility (Kruskal-Wallis, mean rank = 17.1 and 29.6 for high and medium male sterility, respectively, $P = 0.05$). However, the difference in mean rank between low male sterility (mean rank = 26.75) and both medium and high male sterility was not significant at the 0.05 level.

DISCUSSION

The survey results demonstrate that male sterility is uncommon in *C. fasciculata*, and when it does occur in a population it is low in frequency (0–6%). This finding raises the question of why it is uncommon. Several of the factors that determine the maintenance of male sterility in natural plant populations—mode of inheritance, compensation, and pollination system—appear to be important determinants of this low frequency.

Genetic basis of male sterility—The crosses clearly indicate that male sterility in *C. fasciculata* is not purely cytoplasmic in transmission. Progeny of male-sterile mothers showed a mixture of male-sterile and hermaphroditic progeny in both the F_1 and F_2 generations. The frequency of male steriles among the progeny of selfing hermaphrodites also lends no support for a purely cytoplasmic mode of inheritance. If male sterility restorer alleles are specific to a local male-sterile cytotype, then there should be significantly more male steriles in the F_1 and F_2 of interpopulational crosses than in the intrapopulational crosses (Sakai and Weller, 1991; Molina-Freaner and Jain, 1992b). Although male sterility was generally lower in crosses between male steriles and hermaphrodites from GLP vs. male steriles crossed with hermaphrodites from other populations, the differences were not significant. Because of limited sample sizes we cannot completely rule out NCMS, however the low frequency of male sterility observed in natural populations of *C. fasciculata* supports a nuclear mode of transmission.

The high frequency of male sterility from selfed hermaphrodites implies that male sterility in *C. fasciculata* may be caused by the expression of homozygous recessive alleles following selfing. We have also frequently observed male steriles in progeny derived from selfed crosses in other experiments (Fenster, personal observation). Male steriles were observed in the F_1 of both interpopulational and intrapopulational crosses with hermaphrodites suggesting that either male sterility is controlled by genes that are partially additive or that there is a high frequency of recessive alleles in populations. Other studies have also documented nuclear inheritance of male sterility in natural populations, of both gynodioecious and subdioecious species: *Cucurbita foetidissima* (Kohn, 1989), *Schiedea globosa* (Sakai and Weller, 1991), and *Phacelia linearis* (Eckhart, 1992a).

The greater frequency of male sterility detected in the field populations and in greenhouse plants flowering during peak summer heat further suggests that the expression of male sterility in *C. fasciculata* may in part be environmentally determined. In sum, there may be various genetic pathways to male sterility in *C. fasciculata* that involve mostly recessive alleles but that have a complex inheritance because of the role of environmental factors.

Compensation—Compensation has long been suggested and documented as a mechanism for maintaining and/or spreading male sterility in plant populations (Darwin, 1877; Lloyd, 1975; Charlesworth and Charlesworth,

1978; Frank, 1989; Kohn, 1989; Couvet et al., 1990; Sakai and Weller, 1991; Atlan et al., 1992; Eckhart, 1992a, b; Sakai et al., 1997). However, we observed no evidence of reproductive compensation in male steriles of *C. fasciculata,* corresponding to the findings of Molina-Freaner and Jain (1992a) in *T. hirtum.* The paucity of flowers on male steriles suggests that they will produce fewer fruit than hermaphrodites and may be at a disadvantage when attracting pollinators (Stanton, Snow, and Handel, 1986). These results suggest that mutations associated with male sterility in *C. fasciculata* do not have the pleiotropic consequence of increasing female function.

The only measured characteristic that showed compensatory value was nonreproductive biomass. Male steriles were found to be nearly one-third more massive, and this may reflect redirection of resources from reproduction to vegetative biomass. For an annual plant like *C. fasciculata,* increased vegetative biomass at the cost of reproductive function is unlikely to lead to increased fitness. Other studies with the perennials *Phacelia linearis* (Eckhart and Chapin, 1997) and *Plantago lanceolata* (Poot, 1997) have also documented that loss of pollen production can lead to increased biomass accumulation and greater nitrogen allocated to vegetative structures. Overall, these results suggest that important compensation effects associated with male sterility may also be expressed in terms of increased future opportunities for reproduction.

Frequency-dependent selection—We found no evidence of a frequency-dependent selection effect on female reproductive success for male steriles in *C. fasciculata.* Male-sterile simulants completely surrounded by hermaphrodites produced no more fruit than did male-sterile simulants surrounded by other male-sterile simulants. This is a surprising result considering the a priori expectation that pollinators could learn to avoid regions in populations that offer no reward, but not individual plants. Furthermore, plants in the male-sterile simulant condition initiated and matured very few fruits and significantly fewer than when in the hermaphrodite condition.

The bumble bee visitation data demonstrate that bumble bees visited areas of high (100%) male sterility less often than they did the other two areas. At first glance this appears to support a frequency-dependent effect for male steriles. However, data were not collected on the pattern of pollinator movement within individual regions of male sterility. Several studies have shown that bumble bees have a strong tendency to remain within a patch of flowers/plants that offer nutritive rewards (Heinrich, 1979; Schmitt, 1980; Kato, 1988; Dukas and Real, 1993) and that if rewarded by one flower/plant are likely to visit immediate neighbors (as evidenced by their short flight distances between foraging bouts). In our study, the nearest neighbor of a male-sterile simulant was likely another hermaphrodite in the areas of low-frequency male sterility. Thus, the higher frequency of visitation in low and medium male sterility compared to the area of high male-sterile frequency that we observed in our experimental array may simply reflect frequent pollinator visitation to neighboring hermaphroditic, not male-sterile simulants. The lack of frequency-dependent effect may have also been influenced by the size of the artificial array. The probability that a bee adequately visited a male-sterile simulant when in low frequency may have been influenced by their previous experience in the nearby high-frequency male-sterile area.

The pollinator visitation data demonstrate that bumble bees visited plants in the experimental array, but they either avoided the male-sterile simulants or spent very little time on them. Similarly, in another buzz pollination system both solitary and social bumble bees were able to assess pollen rewards while visiting *Solanum* flowers (Buchmann and Cane, 1989). Bees stayed on flowers for a shorter time when pollen had been previously harvested or when pollen removal was blocked by the application of glue to the terminal apertures of the anthers. Pollinator visitation rates are also reduced in females of dioecious *Antennaria parviflora* (Bierzychudek, 1987) and gynodioecious *Hebe stricta* (Delph and Lively, 1992) compared to their male or hermaphroditic counterparts that supply pollen reward. In the former case, seed set is pollen limited and is less than in related plants which reproduce seed asexually. Under laboratory conditions bumble bee scent marks flowers that are good sources of nectar (Schmitt and Bertsch, 1990) and some evidence of this behavior has been claimed for plants in the field (Kato, 1988). If bumble bees also do this for pollen foraging, it is possible that the flowers of the hermaphrodites were marked because they were good candidates for pollen foraging, thereby promoting further visitation by bumble bees. This would reduce the number of fruits produced by male-sterile simulants compared to hermaphrodites.

Hermaphrodites might be expected to have a higher fruit-to-flower ratio than male steriles because *C. fasciculata* hermaphrodites can self through geitonogamy. Previous work has shown that *C. fasciculata* has approximately a 20% frequency of selfing (Fenster, 1991a). However, if we extrapolate outcrossing rates from natural populations to our artificial array of plants, we conclude that selfing only accounts for a fraction of the difference in fruit production between the hermaphrodites and male-sterile simulants.

Frequency-dependent selection has been suggested as a mechanism for maintaining both male steriles and hermaphrodites in natural plant populations (Lloyd, 1975; Molina-Freaner and Jain, 1992a). It appears, however, that male sterility in *C. fasciculata* is not affected by frequency-dependent selection and that this type of selection does not play a role in maintaining male sterility in this species. This is a surprising result given that frequency-dependent effects should play a large role in systems where the unrewarding flowers mimic rewarding flowers of the same species (Dafni, 1984). In these systems, mimics should do well when low in frequency because of mistake pollination, but suffer when high in frequency as compared to the model (in this case, the hermaphrodite plant being imitated) (Weins, 1978; Barrett, 1987). The absence of frequency-dependent effects for male sterility in *C. fasciculata* may be based solely on the structure of the pollination systems. Bawa (1980) and Charlesworth (1993) suggested that highly discriminating and specialized pollinators may be negatively correlated with unisexual flowers. Any compensatory advantages imparted by being female may have costs because of re-

duced pollinator visitation. Bumble bees may be highly adept at scent marking and perceiving rewarding flowers (thereby making them highly discriminating) when compared to other insect pollinators. Consequently, very few male steriles of *C. fasciculata* would be pollinated (irrespective of their frequency in a patch or in the population) and male sterility should be selected against, if compensation and inbreeding depression are minimal. These results suggest that where pollen is the sole reward, male sterility may actually be a constraint to the evolution of dioecy because male steriles may simply suffer too great a failure in female reproductive success. A recent survey by Renner and Feil (1993) appears to support this. They demonstrate that deceit pollination is common in tropical dioecious angiosperms. However, where pollen is the reward in these deceit pollination systems, nonspecialists are the primary pollinators. Furthermore, where dioecy has evolved in pollen reward systems, females (= male steriles) often provide alternative rewards, e.g., sterile pollen (Anderson, 1979; Kevan and Lack, 1985).

Persistence of male sterility—Fenster (1991b) found inbreeding depression to be severe in *C. fasciculata*. However, since outcrossing is so high in *C. fasciculata* (80%) it does not appear to be a factor generating a twofold increase in the fecundity of male steriles, as is necessitated by nuclear inheritance. As described here, frequency-dependent selection and compensation cannot alone explain the maintenance of male sterility in *C. fasciculata* at low frequency. The question of what causes the observed frequency of male sterility (0–6%) in populations of *C. fasciculata* remains. One possible explanation is that pollen production may be a highly mutable trait and the observed frequency may simply reflect selection–mutation balance at many loci. Thus, there may be a frequent recurrence (instead of a maintenance) of male sterility in *C. fasciculata* populations.

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