

ENVIRONMENTALLY INDUCED VARIATION IN FECUNDITY COMPENSATION IN THE MORPH-BIASED MALE-STERILE DISTYLOUS SHRUB *ERYTHROXYLUM HAVANENSE* (*ERYTHROXYLACEAE*)¹

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The evolution of male-sterile individuals in hermaphroditic species represents the first step in the evolution of sex specialization. For male-sterile individuals to persist they must have some fitness advantage that compensates for their loss of the male function. Female fecundity also depends on environmental factors as those determining the likelihood of pollination and fertilization. Here we assessed the effects of both male sterility and reproductive synchrony (an environmentally affected trait) on the magnitude of female compensation of *Erythroxylum havanense*, a distylous shrub with morph-biased male sterility. In vitro measurements of pollen germination showed that thrums were more male sterile than pins. The compensatory advantage of thrums changed by a factor of five depending on flowering synchrony. Flowering in synchrony with the population increased fruit production in both morphs. However, because pins that flowered out of synchrony produced almost no fruits, the reproductive compensation of thrums was higher in these circumstances. Because the magnitude of compensation is frequently considered as a key factor in the evolution of sex specialization, the environmentally induced variation in the magnitude of the reproductive compensation of thrum plants may have profound effects on the evolutionary dynamics of the reproductive system of *E. havanense*.

Key words: compensatory advantage; distyly; Erythroxylaceae; *Erythroxylum havanense*; flowering synchrony; Mexico; morph-biased male-sterility.

Ever since Darwin (1877) the evolution of sex specialization has been a subject of deep interest among evolutionary biologists. The presence of male sterility in hermaphroditic plants has been considered a key factor influencing the evolution of sexual specialization (Lloyd, 1975; Charlesworth and Charlesworth, 1978; Couvet et al., 1990; Maurice et al., 1993; Schultz, 1994; Webb, 1999), because both theoretical and empirical evidence point out that male sterility in hermaphroditic species may represent the first step in the evolution of dioecy (Kohn, 1988; Weller and Sakai, 1991; Charlesworth, 1991; Mayer and Charlesworth, 1992; Geber et al., 1999; Gigord et al., 1999). Because hermaphroditic plants achieve roughly half of their fitness through the male function, male sterility results in a reproductive disadvantage. Consequently, for females to be maintained within a population, male-sterile individuals must compensate for the fitness loss through the male function (Charlesworth, 1981; Gouyon and Couvet, 1987; Delph et al., 1999). Several theoretical studies have shown that the magnitude of compensation required to maintain male-sterile individuals in the population is determined by the mode of inheritance of male sterility. In the case of nuclear inheritance, females must be more than twice as fecund as hermaphrodites (Ross and Gregarious, 1985; Frank, 1989; Gouyon et al.,

1991). When male sterility is due to a cytoplasmic mutation, females will spread in the population with only a slight reproductive advantage (Lewis, 1941; van Damme, 1983; Belhassen et al., 1991; Koelwijn and van Damme, 1995). If both nuclear and cytoplasmic genes are responsible for male sterility (i.e., the inheritance is nucleocytoplasmic), the magnitude of compensation is expected to be intermediate between that expected for nuclear and cytoplasmic inheritances (Lewis, 1941; Charlesworth, 1981; Gouyon and Couvet, 1987).

Although some part of the variation in fecundity between females and hermaphrodites might be associated with the mode of inheritance and the physiological mechanism of compensation (e.g., genetic variation for resource reallocation), it is well known that female fecundity also depends on several environmental factors (pollinators, resource and pollen limitation, reproductive phenology, seed predation, population sex structure, and metapopulation dynamics). For example, female plants of the gynodioecious *Phacelia dubia* had higher fruit and seed set than hermaphrodites after hand-pollination, but no evidence of compensation was observed in natural conditions (Del Castillo, 1993). Such a result suggests that ecological factors might have a strong influence on the relative fecundities of females and hermaphrodites in natural conditions. Nonetheless, studies evaluating the magnitude of compensation in gynodioecious species usually assume that mating opportunities are equal for all plants within a population, and that the magnitude of female compensation depends exclusively on post-pollination mechanisms. It is therefore reasonable to expect that the extent of compensation could be influenced by ecological factors, particularly those determining the likelihood of pollination and ovule fecundation. For instance, even though male-sterile plants have the ability to produce more or better seeds, pollen or pollinator limitation could reduce the advantages of compensation. In accordance, given

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that the magnitude of female compensation of male-sterile individuals has been considered as a key factor in both the maintenance of gynodioecy and the evolution of dioecy (Gouyon et al., 1991; Maurice et al., 1993, 1994; Schultz, 1994), the influence of environmental variance in the expression of compensation could affect the tempo and mode of the evolutionary trajectory of sex specialization in species with male sterility. Few studies, however, have taken into account the combined effects of male sterility and ecological factors on the magnitude of female compensation (but see Williams and Fenster, 1998; Graff, 1999; Taylor et al., 1999; Marshall and Ganders, 2001; Ramsey and Vaughton, 2002).

In this study we evaluated the effects of both male sterility and reproductive synchrony (a factor influencing pollinator attraction and the availability of mates) on the magnitude of female compensation in a natural population of the distylous shrub *Erythroxylum havanense* Jacq. (Erythroxylaceae), a species with morph-biased male sterility (Domínguez et al., 1997). A study involving five populations showed that pin individuals produce on average 49% more viable pollen than thrums (Cuevas et al., in press). Results from hand-pollination experiments showed that male-sterile thrum plants may have a strong compensatory advantage. The probability to survive to age 8 and reproduce associated to the progeny derived from thrum mothers was 0.092, while this value amounted 0.033 for the progeny derived from pin mothers (a 2.8 reproductive advantage; Avila-Sakar and Domínguez, 2000). Although this advantage is expressed after hand-pollination, we have noticed that the magnitude of compensation under natural conditions may show a striking variation among reproductive seasons (Domínguez et al., 1997; Avila-Sakar and Domínguez, 2000; C. A. Domínguez, personal observation).

On the other hand, it has been shown that female fitness in *E. havanense* is highly influenced by flowering synchrony (Domínguez and Dirzo, 1995). Flowering phenology of *E. havanense* is characterized by a marked intra- and inter-individual flowering synchrony. Selection analyses showed that flowering time explains 20–27% of the individual variance in female fecundity, indicating that fitness is highly dependent on flowering synchrony (Domínguez and Dirzo, 1995). Selection gradients on flowering time for 1987 and 1988 were opposite in sign, indicating the presence of a heterogeneous regime of natural selection. It was further demonstrated that such heterogeneity was a consequence of the annual variation in the patterns of rainfall (Domínguez and Dirzo, 1995).

Hence, *E. havanense* is a species in which mate availability and/or mating likelihood depend on flowering synchrony, an attribute highly influenced by environmental variance (Domínguez and Dirzo, 1995). Moreover, male-sterile thrum individuals show a marked compensatory advantage after hand-pollination (Avila-Sakar and Domínguez, 2000), but whose magnitude varies from year to year in natural conditions (Domínguez et al., 1997). This situation offers the opportunity to evaluate whether or not the expression of compensation in male-sterile plants depends on flowering synchrony. In this study we evaluated the joint effects of male sterility, flower production, and flowering synchrony on the female reproductive success of individuals from a natural population of *Erythroxylum havanense* from the Pacific coast of Mexico.

MATERIALS AND METHODS

Study site—The study was carried out at the Chamela Biological Station located on the Pacific coast of the state of Jalisco, Mexico (19°30' N, 105°03'

W). This site is characterized by a marked dry season from November to May, with 80% of the precipitation falling from July to October (Bullock, 1986). The vegetation is a dense tropical deciduous forest. A detailed description of the flora and climate of the Chamela region can be found in Bullock (1986), Lott et al. (1987), and Noguera et al. (2002).

Study plant—*Erythroxylum havanense* is a perennial distylous shrub with complete intramorph incompatibility (i.e., no fruits are produced from crosses within the same morph; Domínguez, 1990). Plants grow on patches of 20–80 individuals. Flowering initiates 7–10 d after the first intense rains that mark the beginning of the wet season when individual flowering thresholds are reached by the increase in soil humidity (Domínguez and Dirzo, 1995). Once flowering is triggered, individual plants flower for a very short period (2.8 ± 0.1 d, mean ± 1 SE hereafter). The availability of water for plants depends on a series of highly heterogeneous environmental factors as topographic zone (slopes, plateaus or gullies, aspect; Domínguez and Dirzo, 1995), soil characteristics (texture, organic matter content, infiltration; Domínguez and Dirzo, 1995; Cotler et al., 2002), and rainfall heterogeneity (García-Oliva et al., 1991; García-Oliva et al., 2002). All these environmental attributes interact with each other to produce a complex spatio-temporal dynamics in terms of water availability for plants. Accordingly, flowering phenology may range from synchronous years characterized by short flowering periods (5.7 ± 0.9 d) in which 84% of the total flower production occurs in two peak flowering days; to asynchronous years with relatively extended flowering periods (up to 20 d) and a more evenly distribution of flower production (Domínguez and Dirzo, 1995). Flowers are small (~1 cm of diameter), and stigma receptivity is limited to the first half of the day. *Erythroxylum havanense* is mainly pollinated by wild bees of the genus *Trigona* (Apidae) and *Xylocopa* (Anthophoridae) (Domínguez et al., 1997). Fruits are ovoid red drupes (1 cm long) that contain only one fertile ovule, and thus are single seeded. As mentioned above, *E. havanense* is also characterized by morph-biased male sterility associated with a compensatory female advantage in thrum plants (Domínguez et al., 1997; Avila-Sakar and Domínguez, 2000). The magnitude of compensation, however, shows a marked temporal variation among reproductive seasons.

Data collection—**Reproductive phenology**—During the reproductive season of 2000 we followed the flowering and fruiting phenology of all the reproductive plants from the Ardilla population ($N = 76$). For each plant we made daily censuses to record the number of flowers, immature fruits, and ripe fruits. Censuses were initiated the first day a plant produced flowers (7 June 2000) and finished when all fruits in the population had ripened (12 July 2000).

Flowering synchrony—Flowering synchrony in *E. havanense* had been previously measured as the inter-individual variation in flowering initiation day (see Domínguez and Dirzo, 1995). Nonetheless, the extreme flowering synchrony we observed in 2000 (see Results) precluded the usage of this index. Instead, we used an index that accounted for the small differences in the timing of flower production that we observed among the individuals from the Ardilla population in 2000. For each plant we estimated the proportion of its flowers that were produced during the peak flowering day of the population. This value ranges from 1 to 0. Plants with values (synchrony index) close to 1 produced a high proportion of their flowers when a large amount of pollen and ovules were available in the population. Values close to zero indicate plants that produced most of their flowers out of synchrony with the population.

Male sterility—To assess the amount of variation in male sterility, as well as to estimate the repeatability of this attribute, we made in vitro pollen cultures during the reproductive seasons of 1998 and 1999 for the same 24 plants. In the 1999 sample, 23 new plants were included for a total of 47 individuals. One day before anthesis several branches on each plant were bagged to prevent pollinator visitation and contamination with pollen from other plants. Pollen collections were made on the day of anthesis between 0900 and 1200. We collected four flowers per plant, and each of four anthers per flower was

rubbed onto the surface of previously prepared culture medium on a microscope slide. The medium was modified from Mazer (1987) and was composed of 250 g/L of sucrose, 0.238 g/L of H_3BO_3 , 0.357 g/L of $Ca(NO_3)_2 \cdot 4H_2O$ and 8.33 g/L of agar. Previous experiments have shown this culture medium is adequate for the evaluation of pollen viability in *E. havanense* (Avila-Sakar and Domínguez, 2000; Cuevas et al., in press). Time between pollen collection and initiation of cultures was always less than 5 min. Cultures were incubated in Petri dishes at ambient temperature, and after 12 h of growth they were fixed with FAA (formaldehyde (37–40%), ethyl alcohol (95%), glacial acetic acid, and water; 10 : 50 : 5 : 35 v/v) and sealed with clear nail polish. Using an optical microscope (Olympus model BH 2, with 40 \times optical magnification) we counted the number of germinated and nongerminated pollen grains on every slide. For each plant we calculated the fraction of germinated to total pollen grains from the pooled data (16 slides), and this measure was used as an estimation of the level of male sterility per plant.

Data analysis—Male sterility—We first investigated the effect of floral morph on the level of male sterility by means of one-way analysis of variance (JMP 4.0.4., SAS, 2001). Male sterility was arcsine transformed to improve normality of the error distribution. Because of the difficulty of measuring the heritability (h^2) of male sterility in *E. havanense* (it takes 8 yr for this species to reproduce), we measured its repeatability through the calculation of the interclass correlation coefficient between levels of male sterility in 1998 and 1999 ($N = 24$ plants). Repeatability measures the amount of the phenotypic variance in a trait due to permanent differences between individuals. Given that permanent differences can be attributed both to genetic variance and to the local environment, the repeatability of a trait sets an upper limit to the degree of genetic determination (Falconer and Mackay, 1996).

Effects of morph, flowering synchrony, and flower number on fruit production—In order to evaluate the differences in fruit production between pin and thrum plants taking into account the variance in flower number and flowering synchrony, we performed a covariance analysis (PROC GLM, SAS, 1989). Morph, flowering synchrony, and flower number were included as the main factors in this analysis. We also included the quadratic terms of flower number, and all the interactions among the three main factors. The significance of the morph term should be indicative of compensation if higher fruit production is associated to the more sterile morph. Flowering synchrony measures the dependence of female fitness on the availability of potential mates and on the likelihood of pollination (i.e., the ecological factors). Because flower number has a strong effect on fruit production (Domínguez and Dirzo, 1995), we included this variable within the analysis. Given that flower number and flowering synchrony were measured in very different scales and because we were interested in comparing their relative effects on fruit production, they were standardized to mean zero and standard deviation 1 prior to analysis ($(x - \bar{x})/SD$). This procedure allowed a direct comparison between these variables because they were expressed in units of standard deviations.

RESULTS

Reproductive phenology—Seventy-six individuals flowered in the Ardilla population after the first heavy rains of the 2000 reproductive season. Altogether, plants produced 51 447 flowers in just three days showing a striking inter-individual synchrony. Most flowers (86.75%) were produced during the first day, 12.95% in the second, and the remainder during the third day. Both pin and thrum plants showed high average values of flowering synchrony (0.81 ± 0.04 and 0.75 ± 0.03 , respectively) and there were no statistical differences among them ($F_{1,75} = 1.06$, $P = 0.31$). Flower production was highly variable among individuals (range 1–4194 flowers, 668 ± 207 flowers). There were no differences between thrum and pin individuals in flower production (771 ± 275 and 532 ± 318 flowers, for thrum and pin plants, respectively; $F_{1,75} = 0.32$, $P = 0.57$).

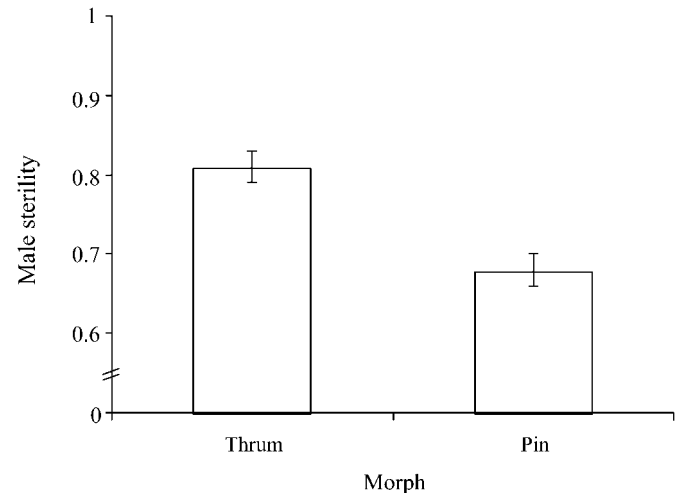


Fig. 1. Average values (± 1 SE) for the in vitro estimations of the levels of male sterility for pin and thrum plants of *Erythroxylum havanense* from the Ardilla population.

Male sterility—Male sterility ranged from 0.43 to 1, with a mean of $0.75 (\pm 0.019)$. Preliminary paternity analysis for the same population investigated in this study showed that plants with levels of male sterility higher than 0.9 sire not a single seed; thus, they can be considered completely male sterile (F. Rosas and C.A. Domínguez, unpublished data). Accordingly, 14% of the plants in the Ardilla population were completely male-sterile. Thrum plants had a higher proportion of completely male-sterile plants (0.23) than pins (0.05), and also a higher level of male sterility (0.81 ± 0.02 and 0.68 ± 0.02 , for thrum and pin plants, respectively; $F_{1,45} = 13.56$, $P = 0.006$, $R^2 = 0.21$; Fig. 1). The estimated repeatability of male sterility was $0.47 (\pm 0.11)$, indicating that a relatively high fraction of the phenotypic variation in male sterility is explained by permanent differences among individuals ($F_{1,22} = 15.8$, $P = 0.0006$, $R^2 = 0.39$). The significant difference in male sterility between floral morphs, along with its constant expression through time (repeatability), suggests that male sterility in *E. havanense* has a heritable component that is independent of environmental variation.

Effects of morph, flowering synchrony, and flower number on fruit production—Analysis of covariance revealed that all three main factors had a significant effect on fruit production (Table 1). The interactions between flowers \times morph and flowers \times synchrony had also a significant effect (Table 1). The fitted model explained 88% of the variance in fruit production. The significance of the morph term reflects the higher seed production of thrum plants (47.5 ± 11.54 and 88.1 ± 10.2 fruits, for pin and thrum plants, respectively). In accordance with previous results (Domínguez and Dirzo, 1995), flowering synchrony had a positive and significant effect on fruit production. Flower number had also a positive effect on fruit production, simply indicating that plants with many flowers produce more fruits. The significance of the interaction flowers \times morph showed that the slope of fruit production on flower number was higher for thrum plants. Hence, the more male-sterile morph (i.e., thrum) was more efficient to mature fruits as the number of flowers per plant increased (Fig. 2). As indicated by the significance of the interaction between flowers \times synchrony, natural selection favored those plants

TABLE 1. ANCOVA of the effects of morph, flowering synchrony, and flower number on fruit production of *Erythroxyllum havanense* from Chamela, Jalisco, Mexico.

Source of variation	df	MS	F	P
Morph	1	72675.97	20.05	<0.0001
Flowering synchrony	1	161472.39	44.56	<0.0001
Flower number	1	285428.97	78.79	<0.0001
Morph × synchrony	1	647.07	0.1786	0.674
Morph × flower number	1	193429.73	53.38	<0.0001
Synchrony × flower number	1	188463.91	52.01	<0.0001
(Flower number) ²	1	8926.73	2.46	0.12
Error	68	3623.0		

that produced many flowers and flowered in synchrony with the population. The combined effects of flower number and flower synchrony had a dramatic influence on the reproductive compensation of thrum plants. Because compensation in *E. havanense* may be defined as the relative fecundity of thrum vs. pin individuals, and flowering synchrony had a marked influence on the reproductive output of both morphs, the magnitude of compensation changed as a function of an environmentally induced trait. Based on the model adjusted by the covariance analysis, we produced two different scenarios regarding the effect of flowering synchrony on the magnitude of compensation (expected fruit production of pins = 63.6 +

147.99 × flowers + 86.13 × synchrony + flowers × synchrony × 253.9; and expected fruit production of thrums = 128.62 + 385.98 × flowers + 86.13 × synchrony + flowers × synchrony × 253.9). Figure 2A shows the expected fruit production of pin and thrum plants flowering under relatively high flowering synchrony conditions (synchrony index = 0.5 SD above the population mean). This figure also shows the estimated values of compensation under this scenario (fruit production of thrums/fruit production of pins). Figure 2B, in contrast, illustrates plants flowering out of synchrony with the population (synchrony index = -0.5 SD below the population mean). It is clear from these figures that fruit production is highly dependent on both flower number and flowering synchrony, but, unexpectedly, the magnitude of compensation was higher under low synchrony conditions.

Overall, the results of this study indicate that fruit production is maximized when flowering synchrony produces the conditions that favor pollinator attraction and increase the availability of mates. The expression of compensation followed an opposite pattern, because the unfavorable conditions for reproduction produced by low flowering synchrony resulted in a meager fruit production in pin plants, thus increasing the relative fecundity of thrum plants.

DISCUSSION

We have shown in this study that the magnitude of compensation of the thrum morph of *Erythroxyllum havanense* depends on flowering synchrony. Thrum plants, the morph with higher male sterility, produced more fruits per flower than pin individuals of equivalent size. Flowering synchrony had also a dramatic positive effect on fruit production in both morphs. Moreover, the magnitude of the reproductive compensation of thrum plants ranged up to 5 times depending on the level of flowering synchrony. It should be noted that 2000 was an extremely synchronous year (see Domínguez and Dirzo, 1995), and even so we were able to detect an important difference between synchronous and asynchronous plants. Consequently, it could be expected that the effect of synchrony on the reproductive compensation of synchronous vs. asynchronous thrum plants would be even greater in years allowing a higher variation in flowering times (see Domínguez and Dirzo, 1995).

Although we found a marked effect of flowering synchrony on the compensatory advantage of thrums, the direction of such an effect was opposite to our original expectative. Flowering synchrony decreased, instead of increased, the magnitude of compensation. Because pin individuals produced almost no fruits when they flowered out of synchrony, there was an eightfold difference in fruit production between synchronous and asynchronous pin plants. Contrastingly, synchronous

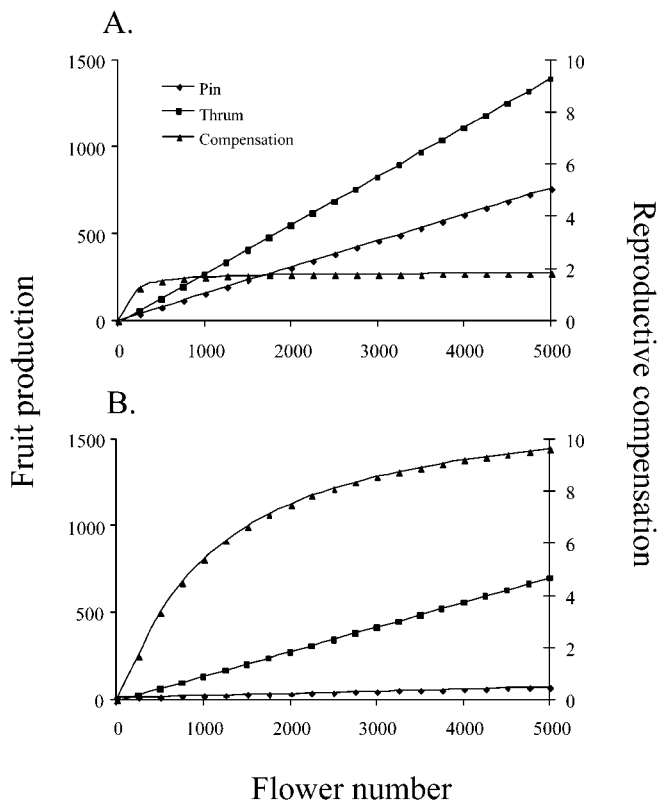


Fig. 2. Adjusted model ($R^2 = 0.88$) describing the relationship between flower number and fruit production for pin and thrum plants of *Erythroxyllum havanense*. Adjustments for plants that flowered in relatively high synchrony (+0.5 SD) with each other (A), and for plants that flowered out of synchrony (-0.5 SD) with the population (B) are presented. The estimated values of the compensatory advantage of thrum plants (fruits of thrums/fruits of pins) are also shown. Plotted values were derived from a covariance model in which we included morph, flowering synchrony and flower number as main factors, as well as the interaction terms.

thrum plants only produced two times as many seeds as thrum individuals flowering out of synchrony (cf. Fig. 2). As a consequence, the magnitude of the compensatory advantage of thrum plants decreased with flowering synchrony. This result should be interpreted with caution because, although the *relative* fecundity of thrums (fruit production of thrums/fruit production of pins) showed an inverse relationship with flowering synchrony, the *absolute* fecundity of both morphs was much higher when plants flowered in synchrony with the population.

The inverse relationship between compensation and synchrony found in this study calls for an explanation. Asynchronous flowering produces a relatively poor reproductive environment (mate, pollen, and pollinator limitation: Augspurger, 1980, 1981; Domínguez and Dirzo, 1995; Domínguez et al., 1997) that reduces the opportunities for successful reproduction of both morphs. Although we actually observed a reduction in fruit production in asynchronous flowering individuals, the negative consequences were more severe on pin than thrum plants. Given that the only difference between the pollen produced by the floral morphs is a higher frequency of abnormal pollen grains in thrum plants (there are no differences in pollen production neither in pollen size, Vázquez-Santana et al., 1996; Domínguez et al., 1997), a load of thrum pollen has a smaller number of viable pollen grains. Hence, pin plants that flowered out of synchrony with the population were exposed to low pollen loads composed of low-quality pollen grains. Thrum plants were also exposed to low pollen loads, but they were pollinated with relatively high-quality pollen. This asymmetry in the qualities of the pollen loads received by each morph could explain the meager fruit production of pin plants and also the elevated value of compensation observed when plants flowered out of synchrony.

Mass flowering has been envisioned as an attribute favored by natural selection because opportunistic pollinators should take advantage of the concentrated amount of rewards that flowering synchrony provides (Gentry, 1974; Augspurger, 1980, 1981; Gross and Werner, 1983; Mori and Pipoly, 1984; Dieringer, 1991). Besides of increasing the attractiveness of a flowering patch to pollinators, flowering synchrony also produces the conditions that maximize the rates of pollen-donation deposition and the opportunities to find mates. This is particularly true for *E. havanense* because this is a self-incompatible species that requires pollinator visitation to produce fruits. In fact, previous studies have shown that flowering in synchrony with the population has marked consequences on the reproductive success of *E. havanense* individuals (Domínguez and Dirzo, 1995).

Accordingly, we think that the differences in the curves describing reproductive compensation as a function of flower number could be explained by the influence of synchrony on the foraging behavior of pollinators. When plants flowered in high synchrony, the curve of compensation reached a plateau at relatively low flower numbers (Fig. 2A). Asynchronous flowering, in contrast, produced a curve in which compensation always increased (Fig. 2B). It is possible that large numbers of flowers confer an important advantage when plants flower out of synchrony, because these plants monopolize most of the few available pollinators. Large pin plants are also expected to attract more pollinators, but because most of the pollen they received is nonviable, their fecundity should be low. When plants flowered in synchrony with the population fruit production increased at a higher rate (number of fruits per flower), but compensation showed an asymptotic behavior

around a value of two. These observations suggest that the high concentration of resources brought about by flowering synchrony reduces the threshold (number of flowers) used by pollinators to discriminate against plants with few flowers. Under these circumstances, both morphs would receive enough viable pollen and fecundity would depend mainly on post-pollination mechanisms.

Obviously the above interpretation depends on the assumption that pollinators discriminate among plants that differ in flower number. Previous studies have shown that the most common species of pollinators of *E. havanense* forage preferentially on plants with many flowers (Domínguez et al., 1997). Moreover, the average fruit set of large plants (those above the mean value of flower number) was significantly higher than that of the small ones (0.41 ± 0.04 and 0.22 ± 0.02 fruits per flower; for large and small plants, respectively; $F_{1,75} = 10.48$, $P = 0.001$), suggesting that pollinator visitation was more intense in the former than in the latter individuals. Pollen limitation has also been proved to affect female fecundity in the gynodioecious *Silene vulgaris* (McCauley et al., 2000), and in *Wurmbea biglandulosa* (Ramsey and Vaughton, 2002).

Results of this study support previous findings (Domínguez et al., 1997; Avila-Sakar and Domínguez, 2000) indicating that the partial loss of the male function of thrum individuals of *Erythroxyllum havanense* is compensated by a fitness increase through the female function. After hand-pollination thrum individuals outperform pin ones as maternal plants by producing more and better seeds (Avila-Sakar and Domínguez, 2000). However, as this study demonstrates, the expression of compensation in natural conditions depends on flowering synchrony, probably because of its effect on successful pollination. As a consequence, the magnitude of the reproductive compensation of thrum plants of *E. havanense* ranged from zero (in small plants) to almost ten (in the larger plants). Reproductive compensation has been described in other species with male sterility (reviewed in Shykoff et al., 2003), and the magnitude of this parameter is thought to be involved in the maintenance of male-sterile individuals and in the evolution of dioecy (Lewis, 1941; Charlesworth and Charlesworth, 1978; Gouyon and Couvet, 1987; Maurice et al., 1993, 1994; Schultz, 1994; Charlesworth, 1999). Theoretical models predict that in the most stringent conditions (nuclear inheritance), male-sterile individuals should exhibit a twofold compensatory advantage to be maintained in the population (Lewis, 1941; Charlesworth, 1999). Nucleocytoplasmic inheritance, in contrast, requires just a subtle fecundity advantage for polymorphism to be maintained (Couvet et al., 1986; Gouyon and Couvet, 1987; Schultz, 1994). Although we do not know the genetic mechanism determining male sterility in *E. havanense*, our results suggest that this attribute has a heritable component (see also Domínguez et al., 1997). Moreover, independently of the particular mechanism of inheritance, the values of compensation observed in this study were very close, or even much higher, than the most stringent theoretical threshold (under nuclear inheritance) for male-sterile individuals to be maintained in the population. This result suggests that the magnitude of the fecundity compensation (seed production, the quantitative compensation; sensu Alonso and Herrera, 2001) observed in *E. havanense* is high enough as to maintain male-sterile individuals in the population. Given that this species also shows a qualitative compensatory advantage (offspring quality; Avi-

la-Sakar and Domínguez, 2000), the overall value of compensation should be far beyond the theoretical threshold.

Our finding of environmentally induced variation in the expression of the reproductive compensation of *E. havanense* suggests that the selective dynamics of male sterility may change on an annual basis depending on the level of flowering synchrony (Domínguez and Dirzo, 1995). Highly synchronous years would allow maximum fruit production and a relatively low compensatory advantage. Nonetheless, such an advantage is high enough as to favor the permanence of male-sterile individuals. On the other hand, because of the disproportionate effect that pollinator limitation and the scarcity of potential consorts (see Williams and Fenster, 1998; Taylor et al., 1999; Ramsey and Vaughton, 2002) have on the quality of the pollen loads received by pin plants, the compensatory advantage of thrums is boosted during years of low flowering synchrony. Thus, in the particular case of *E. havanense*, male-sterile individuals always enjoy a compensatory advantage whose magnitude changes dramatically depending on flowering synchrony. Given that male sterility in natural populations is considered a key factor in the evolution of sex specialization in hermaphrodite species (Lloyd, 1975; Charlesworth and Charlesworth, 1978; Maurice et al., 1993; Shultz, 1994; Webb, 1999), the potentially intense influence of environmental variance in the expression of compensation could affect the rate of evolution of the reproductive system of *E. havanense*. In general terms, the findings of this study suggest that environmental heterogeneity has the potential to affect the expression of the compensatory advantage of male-sterile individuals in gynodioecious species and, therefore, the evolutionary trajectory of sex specialization.

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