Reproductive consequences of clonal growth in *Stenocereus eruca*, a rare clonal cactus of the Sonoran desert

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Abstract. *Stenocereus eruca* is a prostrated, self-incompatible cactus endemic to the Sonoran Desert that regenerates primarily through clonal propagation. Clonal growth is expected to affect mate availability by influencing the number and spatial distribution of mating types. In this paper we examine the role of clonal growth on female fecundity through a series of pollination experiments in a population of *S. eruca*. We set up a pollen supplementation experiment using five distance treatments with pollen collected at 1, 10, 100, 1000 and 25000 m from receptor flowers during the years 2001 and 2002 and evaluated genetic sifmilarities between pairs of receptor-donor ramets through RAPD markers. Our data on fruit set, number of seeds/fruit, germination and overall fecundity revealed that *S. eruca* show a significant reduction in female fecundity when pollination occurs between ramets located at short distances (1 and 10 m), while genetic data showed high levels of similarity at those distances. The reduction in female fecundity is apparently a consequence of geitonogamy and inbreeding depression. Our data suggest that clonal growth and geitonogamy are likely to be partially responsible for the low levels of sexual reproduction and seedling recruitment observed in populations of *S. eruca*.

Key words: Clonal growth, Geitonogamy, Sexual reproduction, Sonoran Desert, *Stenocereus eruca*

Introduction

Plant self-incompatibility (SI) systems are genetic mechanisms that prevent self-fertilization in hermaphrodites through recognition and rejection of pollen expressing the same allelic specificity expressed in pistils (de Nettancourt, 2001). Theoretical models predict a large number of alleles at the S locus that are maintained by frequency dependent selection (Castric and Vekemans, 2004)

and empirical studies have corroborated those predictions as both gameto-phytic and sporophytic SI systems posses a large number of alleles and mating types within populations (Lawrence, 2000). A key feature of SI systems is the proportion of compatible matings that occur in a population (i.e. mate availability, Vekemans et al., 1998). The type of SI system, population size and clonal growth are known to influence mate availability in plants (Byers and Meagher, 1992; Vekemans et al., 1998; Charpentier, 2002).

Clonal plant populations usually exhibit low fecundity and seedling recruitment (Eriksson, 1993) that are often attributed to pollinator limitation or environmental variation (Jones and Gliddon, 1999; Verburg et al., 2000). However, clonal growth may have a reproductive cost by affecting mate availability through its influence on the number and spatial distribution of mating types in plants (Wang et al., 2005). Low population size often restricts the number of clones and as a consequence fecundity is limited by deficit of compatible outcross pollen (Charpentier et al., 2000). On the other hand, if pollen dispersal is limited to adjacent ramets, high rates of geitonogamous pollination (Handel, 1985; Trame et al., 1995) or non-optimal crossing distances (Waser and Price, 1989) are expected under a clumped distribution of genotypes whereas high rates of outcross pollination are expected under an intermingled distribution (Charpentier, 2002). Thus, in self-incompatible clonal plants with a clumped distribution or small population size, geitonogamy may have a cost by reducing fecundity due to stigma saturation, abortion of self-pollinated ovule and waste of pollen (Charpentier, 2002).

The Cactaceae is a large family that contains numerous species that exhibit self-incompatibility (Boyle, 1997). Members of this family exhibit features like tricellular pollen and papillate stigmas with scant or no stigmatic exudates (Ganders, 1976; Boyle, 1997) that are typically associated with sporophytic SI systems (Brewbaker, 1967). However, evidence from diallel crosses in three genera indicates that the SI system is gametophytic and controlled by a single multiallelic locus S (Boyle, 2003). Thus, the evidence from pollination treatments in numerous groups suggests that SI is widespread in the family (Boyle, 1997) and formal diallel crosses indicate gametophytic control (Boyle, 2003). Many cacti are known to combine sexual reproduction and clonal propagation as mechanisms of regeneration (Bravo-Hollis, 1978; Parker and Hamrick, 1992). However, nothing is known about the interaction between clonal growth and sexual reproduction in clonal cacti.

Stenocereus eruca is a rare, self-incompatible clonal cactus endemic to the Sonoran Desert (Turner et al., 1995; Clark-Tapia and Molina-Freaner, 2004). It is a narrow endemic, restricted to the Plains of Magdalena in Baja California Sur (Fig. 1). Evidence on clonal diversity within and among populations indicate that regeneration occur both through clonal propagation and sexual recruitment (Clark-Tapia et al., 2005a, b). The spatial distribution of genotypes
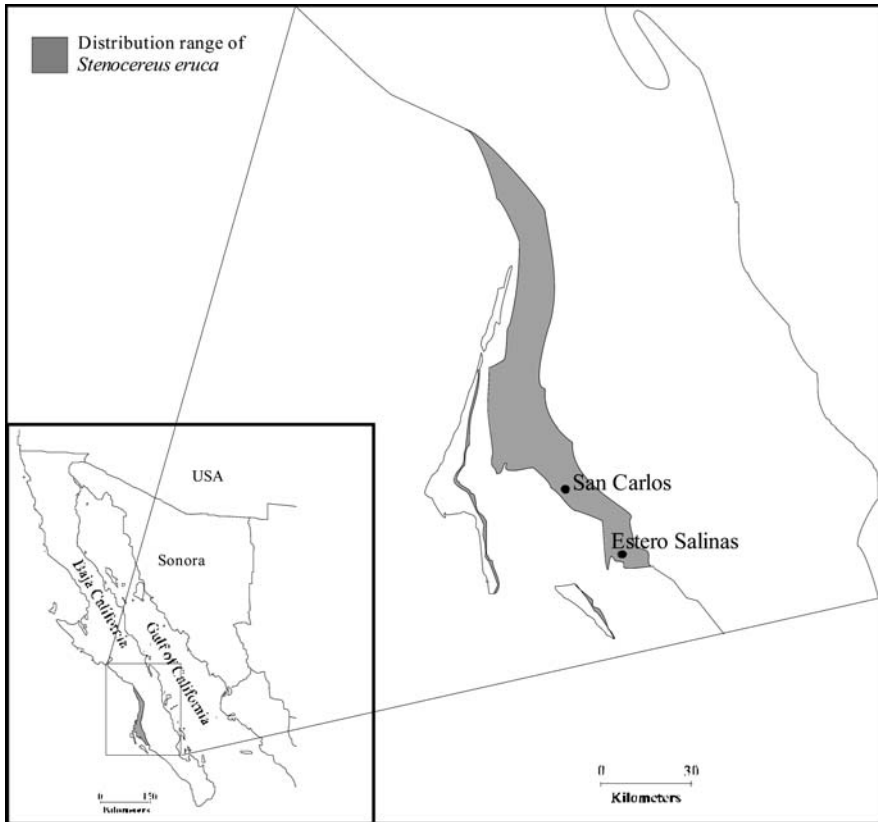
Figure 1. Distribution range (shaded area) of *Stenocereus eruca* in Baja California Sur and the location of the studied populations. Modified from Turner *et al.* (1995).

is clumped with most ramets occurring within 10 m and few clones have a wide distribution within populations (Clark-Tapia *et al.*, 2005a, b; Molina-Freaner and Clark-Tapia, 2005). Fruit set is generally low (0.03–0.15) presumably as a consequence of pollinator limitation (Clark-Tapia and Molina-Freaner, 2004). Major pollinators include Sphingid moths and native bees (Clark-Tapia and Molina-Freaner, 2004). Sphingids are effective pollinators that transport pollen usually at distances greater than 10 m. However, during 4 years of observation, sphingids were observed only during one year when no evidence of pollinator limitation was detected. In contrast, native bees are ineffective pollinators that move pollen usually at distances shorter than 10 m and were observed in years where pollinator limitation was detected. The available evidence indicates that female fertility in *S. eruca* is low as a consequence of spatio-temporal variation in pollinator abundance (Clark-Tapia and Molina-Freaner, 2004). However, nothing is known about the role of clonal growth on female fertility.

In this paper we explore the influence of clonal growth on fruit and seed set through a series of pollen supplementation experiments using pollen donors of contrasting distances. We also explore the role of genetic similarity on the success of crosses by examining the percentage of shared bands in RAPD profiles from pairs of pollen donors and receptors.

Materials and methods

Our pollen supplementation experiments were carried out at Estero Salinas in the Plains of Magdalena, Baja California Sur, Mexico (Fig. 1). *S. eruca* covers an area of approximately 400 ha in this site. We selected this population because in previous studies it is the only one that flowers on a regular basis and it has large number of ramets per unit area (≈3000 ramets/ha; Clark-Tapia and Molina-Freaner, 2004).

Pollen supplementation experiments

These experiments were carried out from August to December in 2001 and 2002, during the flowering and fruit maturation period of this species. In order to examine the effects of distance from pollen donors on fruit and seed set, 60 and 80 reproductive ramets were randomly selected in the population during 2001 and 2002, respectively. In August of 2001 and 2002, flowers from each ramet were manually pollinated with pollen coming from donors located at five distances (1, 10, 100, 1000 and 25,000 m.). The source of pollen from a distance of 25,000 m was another population located north of Estero Salinas, in San Carlos (Fig. 1).

The minimum number of flowers used in each pollen supplementation distance treatment was 10 during 2001 and 20 during 2002. Given that seasonal flower production per ramet is low (Clark-Tapia and Molina-Freaner 2004) only one flower per ramet was employed for the distance treatments. Receptor flowers were distributed along the population at distances of 50–100 m. Receptor flower buds were covered with mesh bags prior to opening and after pollination; flowers in each treatment were covered again in order to avoid visits by pollinators and predation. Forty-five days after the pollination experiments, mature fruits were collected and the success of each treatment was evaluated by: (a) fruit set (proportion of flowers that set fruit), (b) average number of seeds/fruit, (c) total fecundity (fruit set multiplied by the number of seeds/fruit) and (d) germination percentage from each treatment. Seeds came from different ramets within treatments. Germination was evaluated using 7 replicates of 100 seeds per treatment using 2% agar in Petri dishes placed in a growth chamber for 3 months at 25 °C. Seeds in each petri dish were obtained

from bulk samples from all ramets within treatments. We also included a control treatment using seeds from natural pollination in order to evaluate whether their germination rates differ from those obtained in the pollen supplementation treatments.

Genetic identity analysis

In order to examine the level of genetic similarity between pollen donors and receptors, we collected a sample of stem chlorenchyma from every ramet involved in the pollen supplementation experiment. The procedure for the collection, preservation and genetic analysis of the samples using Random Amplified Polymorphic DNA (RAPDs) was the same used in a previous study of clonal diversity (Clark-Tapia *et al.*, 2005a). The genetic similarity between pairs of ramets (pollen donor and receptor) was evaluated by counting the number of shared bands (from a total of 44 loci) among the amplification products with six RAPDs primers. The number of shared bands was used to calculate Nei and Li's (1979) genetic similarity index as $S_G = 2m_{xy}/(m_x + m_y)$, where m_{xy} is the number of shared bands between pairs of ramets, and m_x and m_y are the total number of bands present in each sampled ramet. This measure of genetic similarity allowed us to infer whether pairs of ramets belonged to the same or to a different genet. Pairs of ramets with the same banding profile were considered as belonging to the same genotype, as the random probability of matching profiles was very low (1.8×10^{-8}) given the large number (44) of loci employed.

Data analysis

Fruit set among pollen supplementation treatments were evaluated through χ^2 tests, while one-way ANOVA was used to test for differences in number of seeds per fruit and overall fecundity. Germination data were arcsine transformed and analyzed through a one-way ANOVA and contrast tests (Tukey) were used to identify differences among pollination treatments. Linear regression analysis was used to explore the relationship between geographic distance and genetic similarity, while stepwise regression analysis was used to explore the influence of genetic similarity and distance between pairs of ramets on female fertility. All statistical analyses were performed using the JMP v. 4.

Results

Fruit set varied among the five pollen supplementation distances, from 0 ± 0.00 (mean $\pm$ 1 SD) to 0.80 ± 0.13 and from 0.10 ± 0.07 to 0.68 ± 0.11 in 2001 and 2002, respectively (Fig. 2). During 2001, pollen transfer between

ramets located at distances between 1 and 10 m did not set any fruit, while during 2002 fruit set was 0.10 and 0.23 at those distances (Fig 2). In contrast, pollinations between ramets located at longer distances resulted in significantly greater fruit set during 2001 ($\chi^2 = 22.3$, df $= 4$, $p = 0.0002$) and 2002 ($\chi^2 = 25.9$, df $= 4$, $p < 0.0001$). However, the statistical analysis revealed no significant differences in fruit set values between 100, 1000 and 25,000 m during 2001 ($\chi^2 = 1.64$, df $= 2$, $p = 0.43$) and 2002 ($\chi^2 = 0.19$, df $= 2$, $p = 0.90$).

No fruits were produced by the 1 and 10 m distance treatment during 2001 and therefore no seeds were obtained. The mean number of seeds per fruit varied among distances from 0 ± 0.00 to 251.2 ± 49.32 and from 14.9 ± 12.0 to 244.64 ± 46.57 during 2001 and 2002, respectively (Fig. 2). Pollinations between 1 and 10 m resulted in either no seeds or very low numbers of seeds per fruit while crosses at distances > 10 m produced significantly larger number of seeds per fruit during 2001 ($F = 7.41$, df $= 4$, $p < 0.0001$) and 2002 ($F = 8.57$, df $= 4$, $p < 0.0001$). As with fruit set, no significant differences were detected in seeds per fruit at distances of 100, 1000 and 25000 m during 2001 ($F = 1.11$, df $= 2$, $p = 0.33$) and 2002 ($F = 0.90$, df $= 2$, $p = 0.41$).

Overall fecundity (number of seeds/pollinated flower) among the pollen supplementation treatment distances varied from 0 ± 0.00 to 200.96 ± 39.46 and from 1.49 ± 1.20 to 166.36 ± 31.66 during 2001 and 2002, respectively (Fig. 2). As expected, significant differences were detected among distances during 2001 ($F = 9.73$, df $= 4$, $p < 0.0001$) and 2002 ($F = 12.10$, df $= 4$, $p < 0.0001$).

Germination after 12 weeks varied from 39 to 52% among three pollination distances (100, 1000 and 25000 m) while the control treatment reached values of 28% (Fig. 3). Germination among the 1 and 10 m distance treatments was zero as seeds were usually empty, small or parasitized. The statistical analysis of the arcsine transformed data for all treatments revealed highly significant differences ($F = 333.9$, df $= 5$, $p < 0.0001$). Contrast test revealed no differences between the 1000 and 25,000 m treatment and differences between the control and 100 m treatment (Fig. 3).

The average number of shared bands between pairs of ramets ranged from 33.76 ± 2.83 (mean ± 1 SD) to 28.13 ± 2.99 that translated as actual proportions of shared bands from 0.76 ± 0.08 to 0.62 ± 0.08 for distances of 1 and 25,000 m, respectively. Linear regression analysis detected a significant negative association (Fig. 4) between genetic similarity and geographic distance, both in 2001 ($F = 39.47$, $p < 0.0001$) and 2002 ($F = 35.08$, $p < 0.0001$). Finally, stepwise regression analysis showed that genetic similarity ($F = 64.97$, $p < 0.00001$) and geographic distance ($F = 14.73$, $p < 0.0003$) had a significant effect on fecundity during 2001 (Fig. 5). However, during 2002, stepwise regression analysis showed that only genetic similarity ($F = 52.47$, $p < 0.00001$) had a significant effect on fecundity while geographic distance ($F = 0.038$, $p = 0.84$) had no effect (Fig. 5).
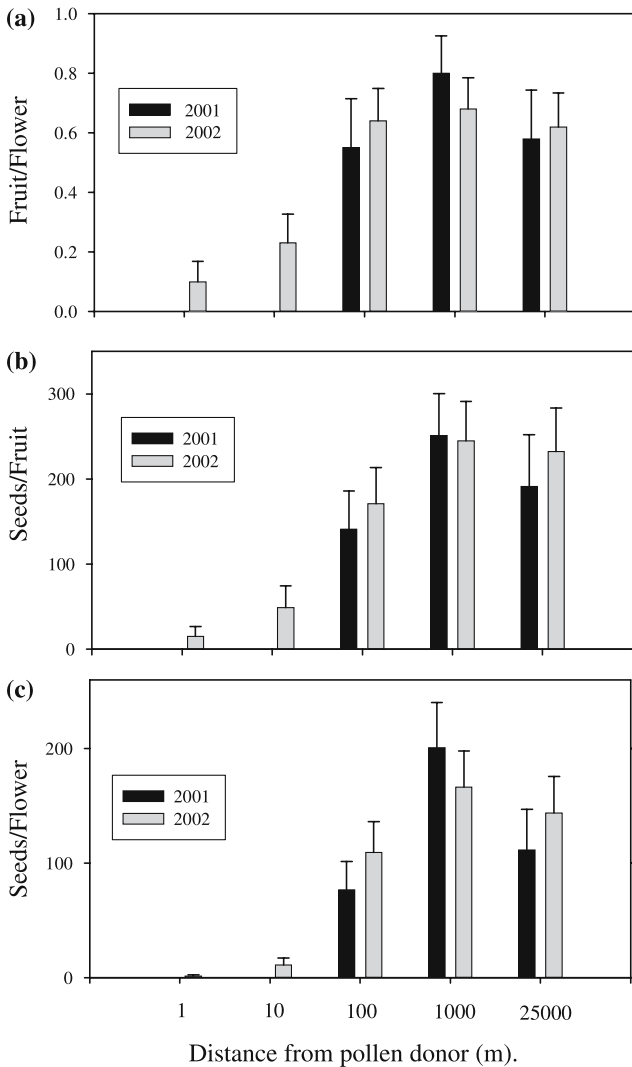
Figure 2. (a) Fruit set (proportion of flowers that set fruit), (b) average number of seeds/fruit, and (c) total fecundity (fruit set x number of seeds/fruit) for the five pollen supplementation treatments (1, 10, 100, 1000 and 25,000 m) in Estero Salinas. Sample size during 2001 was 10 flowers per treatment, while 20 flowers were used in 2002.

Discussion

This study has shown that *Stenocereus eruca* exhibits a significant reduction in fecundity when pollination takes place between ramets that are located at short distances. Most pollinations between ramets located at 1 and 10 m apart failed to produce viable seeds, most likely due to rejection from the self-incompatibility
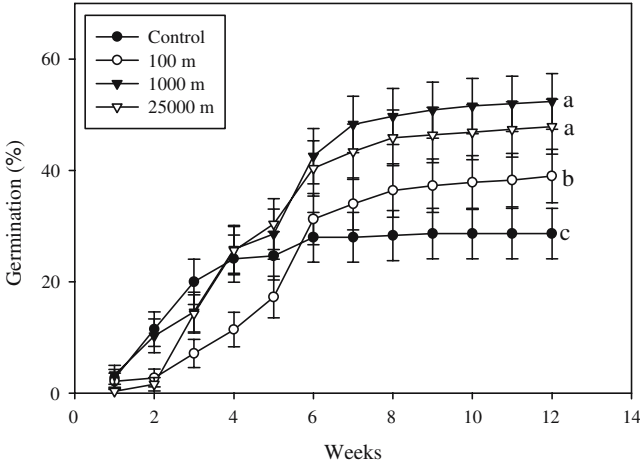
Figure 3. Germination percentage (± 1 SD) for the control, 100, 1000 and 25000 m pollen distance treatments during the first 12 weeks. Different letters indicate significant differences as revealed by contrast tests.

system and inbreeding depression. In contrast, pollen transfer to ramets located at 100 m or longer distances usually produced viable seeds. Genetic similarity declined with distance among ramets, suggesting that genotypes have a clumped distribution that in conjunction with the self-incompatibility system and inbreeding depression are responsible for the reduced fecundity in short distance crosses. Overall, our data suggest that clonal growth has the potential to reduce female fertility if pollinators move pollen at short distances.

Our data on the success of crosses suggest that pollinator movement could have a significant influence on the actual reproductive output of *S. eruca*. Based on flower morphology, the coevolved pollinators of *S. eruca* are thought to be sphingid moths (Gibson, 1989). However, during 4 consecutive flowering seasons, sphingids moths were rarely observed visiting the flowers of *S. eruca* (Clark-Tapia and Molina-Freaner, 2004). In contrast, native bees were regularly observed as flower visitors. Using fluorescent dyes as pollen analogs, native bees were found to move pollen at short distances (< 10 m) whereas sphingid moths were found to transfer pollen at longer distances (Clark-Tapia and Molina-Freaner, 2004). Thus, during years when sphingids are missing and native bees are major flower visitors, *S. eruca* is likely to exhibit low reproductive output. Demographic evidence obtained in four populations observed during four years, showed regular ramet recruitment but no evidence of recruitment of seedlings of sexual origin (Clark–Tapia *et al.*, 2005b). Therefore, the low levels of sexual recruitment that has been observed in *S.eruca* may be due to the low reproductive output that has been detected in most populations (Clark-Tapia and Molina-Freaner, 2004).

Clonal growth is known to affect mate availability by influencing the number and spatial distribution of mating types in plants (Trame *et al.*, 1995;

Charpentier, 2002). The evidence on clonal diversity indicates that populations of *S. eruca* are multiclonal (Clark-Tapia *et al.*, 2005a; Molina-Freaner and Clark-Tapia, 2005). Thus, although it is a narrow endemic species where population size might restrict mate availability (Byers and Meagher, 1992), actual levels of clonal diversity suggest that the number of mating types is not restricted. On the other hand, the evidence on clonal distribution indicates that most genotypes have a clumped distribution while few are widely distributed within populations (Clark-Tapia *et al.*, 2005a, b). Our data on the success of crosses are consistent with the clonal distribution that has been previously detected in *S. eruca*. Our genetic data clearly indicate that genetic similarity
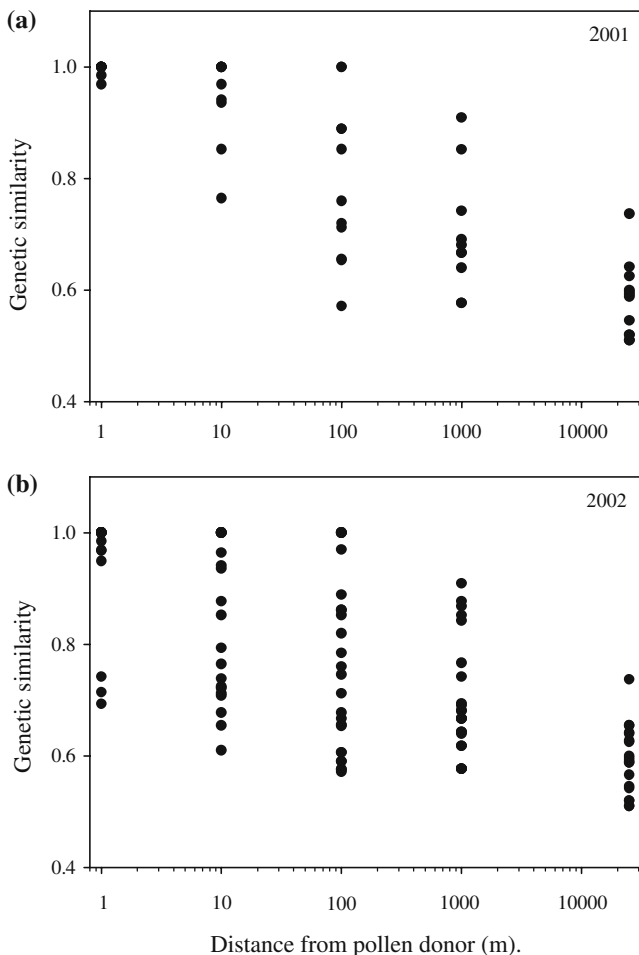

Figure 4. Genetic similarity as a function of geographic distance (m) for pairs of donor-receptor ramets for the pollen supplementation treatments of *Stenocereus eruca* during (a) 2001 and (b) 2002.
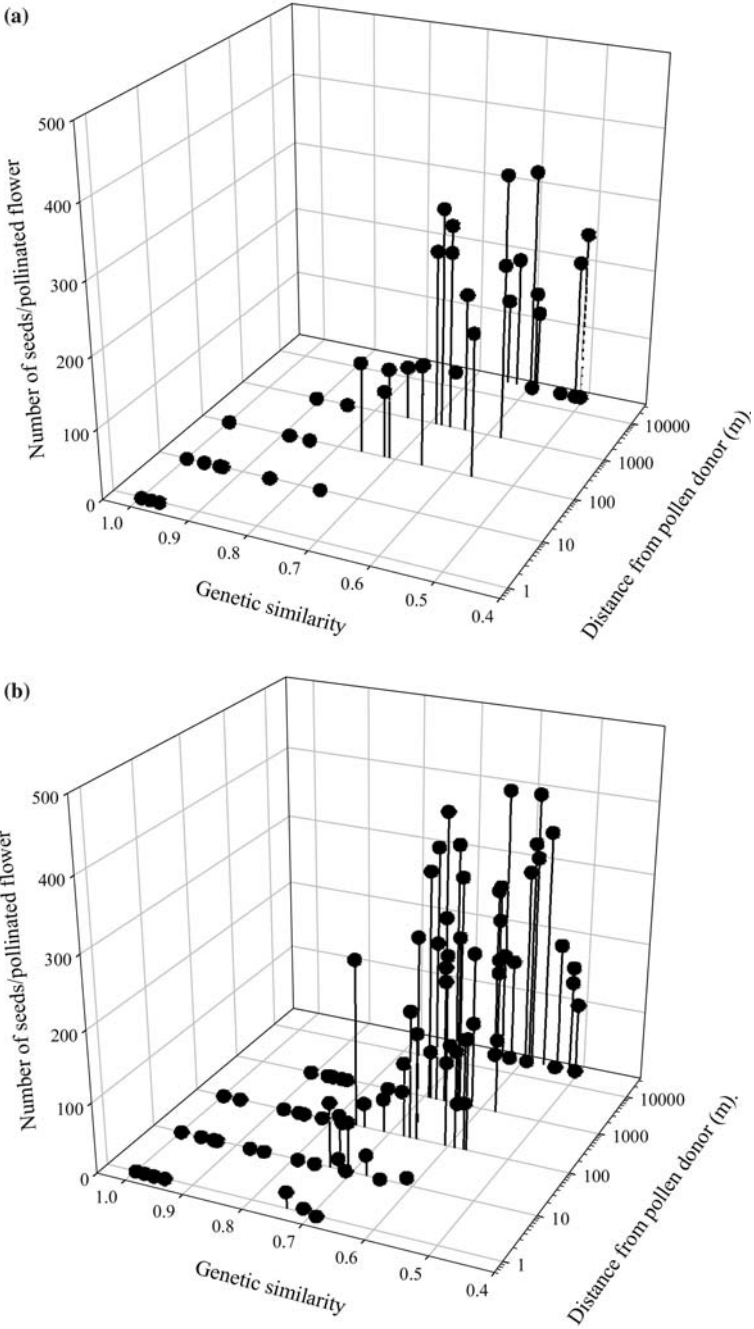
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Figure 5. Female fecundity as a function of genetic similarity and geographic distance for pairs of ramets used in the pollen supplementation treatments of *Stenocereus eruca* during (a) 2001 and (b) 2002.

between pairs of ramets decline with distance. Some pairs of donor–receptor ramets located at short distances shared the same RAPD profile, suggesting that self-incompatibility was responsible for reproductive failure. However, other pairs located at short distances showed high genetic similarity but not an identical genotype, indicating that self-incompatibility was not responsible for the low success of crosses. For those cases, it is likely that inbreeding depression may be the underlying cause for the low reproductive success, as those pairs usually shared a large fraction of bands. A formal comparison of single locus vs. multiloci values of the outcrossing rate (Ritland, 1990) may reveal the contribution of biparental inbreeding in *S. eruca*.

Overall, our data suggest that clonal growth has a reproductive cost in *S. eruca*. The waste of pollen associated with short distance movement may be significant and probably exerts a selective pressure against investment in sexual reproduction. Thus, *S. eruca* may represent a case where sexual reproduction is becoming costly. Clonal propagation may have some short-term ecological advantages for population stability in *S. eruca* and some unknown long-term evolutionary consequences. Future studies should explore the mechanisms involved in the maintenance of sexual reproduction in this rare cactus.

Acknowledgements

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