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"ESTRUCTURA CLONAL, DEMOGRAFÍA Y BIOLOGÍA REPRODUCTIVA DE *STENOCEREUS ERUCA* (CACTACEAE) EN LAS PLANICIES DE MAGDALENA, B.C.S."

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Resumen

Stenocereus eruca es una cactácea clonal que presenta una distribución restringida a las Planicies de Magdalena, en Baja California Sur, y se ha sugerido que sus poblaciones se mantienen predominantemente por propagación vegetativa, debido a que no se han observado plántulas en el campo. En este estudio se describe la estructura clonal y genética, así como la demografía y biología reproductiva de *Stenocereus eruca*. La integración de diversas herramientas en este trabajo permitió: a) evaluar la importancia relativa del reclutamiento vía sexual *vs.* clonal, b) evaluar el comportamiento demográfico a nivel de genets y ramets, así como conocer las causas proximales de los bajos reclutamientos sexuales observados en las poblaciones de *S. eruca*, y c) proponer bases para la conservación de esta especie amenazada. Para evaluar la estructura clonal y genética se utilizó a RAPD´s (random amplified polymorphic DNAs) como marcador molecular. Se utilizó además matrices de proyección tipo Lefkovitch para evaluar la demografía a nivel de genets y ramets durante un período de tres años. Por último, se estudio la biología reproductiva y se realizaron experimentos de suplementación de polen para evaluar si las bajas fecundidades de la especie son producto de una limitación por polinizadores o de una limitación por polen compatible debido a eventos de geitonogamía. Los resultados obtenidos en este estudio, muestran patrones contrastantes en relación con la importancia relativa del reclutamiento sexual *vs*. clonal en las poblaciones de *Stenocereus eruca*. Por un lado, el análisis genético realizado con RAPDS demuestra que las poblaciones de *S. eruca* son multi-clonales, con altos niveles de diversidad genotípica que sugiere reclutamientos sexuales espóradicos, mientras que el análisis demográfico-reproductivo sugiere que el mecanismo de regeneración más importante es la propagación vegetativa en las poblaciones de esta especie. El análisis demográfico de esta especie a nivel de genets y

ramets sugiere que la propagación vegetativa representa un proceso de crecimiento y ocupación del espacio de los genets más que una forma de reproducción, lo cual es un mecanismo que permite a la especie evitar la senescencia e incrementar la adecuación de los genets. Por otra parte, la evidencia obtenida en este estudio sugiere que la limitación por polinizadores, combinada con la estructura espacial de los clones puede ocasionar un gran desperdicio de polen, una disminución en las tasas de germinación y consecuentemente una disminución en la adecuación de *S. eruca*. Con base en la integración de diversas herramientas (genéticas, demográficas y de biología reproductiva), se propone la creación de al menos una reserva Estatal o Federal como una estrategia viable de conservación para esta especie. Finalmente, para lograr una mejor comprensión de los benéficos ecológicos que aporta la clonalidad a largo plazo en especies clonales es necesario estimar las tasas de crecimiento (λ) de genotipos y tasas de entrecruzamiento en semillas.

Abstract.

Stenocereus eruca is a clonal cactus with a restricted distribution in the Magdalena Plains of Baja California Sur that is thought to rely on clonal propagation for population maintenance given that no seedling of sexual origin have been observed in the field. In this thesis, I describe several aspects of the clonal, genetic and demographic structure and the reproductive biology of *Stenocereus eruca*. The objectives were: a) evaluation of the relative importante of sexual *vs* clonal recruitment in population regeneration, b) describe the demographic behaviour of genets and raments and explore the proximal causes of the low sexual recruitment observed in their populations and c) suggest guidelines for the conservation of this threatened species. The clonal and genetic structure was assessed using random amplified polymorphic DNA (RAPD) as molecular markers. Lefkovitch-type projection matrices were employed to describe the demography of genets and ramets during a 3 yr period. Finally, I studied the reproductive biology and used pollen supplementation experiments to explore whether low female fecundities were due to pollinator limitation or due to limitation by compatible pollen associated to geitonogamy events. The evidence concerning the relative importante of sexual *vs* clonal recruitment showed contrasting results. The clonal and genetic structure using RAPD revealed that populations of *S. eruca* are multi-clonal and have high levels of genotypic diversity suggesting that sexual recruitment is important. In contrast, the demographic evidence showed that clonal recruitment was the major mechanism of regeneration. The demographic analysis of genets and ramets suggest that clonal propagation is a mechanism of growth and space occupation by genets rather than a mode of reproduction, thus avoiding senescence and increasing their fitness. On the other hand, pollinator limitation combined with the restricted spatial distribution of genets contribute to pollen wastage,

low seed germination and ultimately to low fitness in *S. eruca*. Based on the integration of different tools and type of evidence, I propose the creation of a State or Federal Reserve for the conservation of viable populations of *S. eruca*. Finally, a better understanding of the long-term consequence of the clonal habit of *S. eruca* require knowledge about the growth rates (λ) of genotypes and outcrossing rates.

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1. INTRODUCCION GENERAL

INTRODUCCIÓN

La reproducción es el mecanismo que permite la perpetuación de las especies a través de una sucesión de generaciones a lo largo de un período prolongado de tiempo (Grand, 1971). Las plantas en particular pueden persistir gracias a diversos mecanismos que involucran la reproducción sexual (i.e. autofertilización o polinización cruzada), así como por medio de la reproducción asexual (i.e. la propagación vegetativa o clonal a través de diferentes estructuras vegetativas tales como estolones, bulbos, rizomas, etc.) (Harper y White, 1974; Richards, 1986; Klimes *et al.*, 1997). Cada forma de reproducción tiene ventajas y desventajas evolutivas particulares sobre una especie determinada. Se ha argumentado por ejemplo, que la principal ventaja evolutiva de la propagación vegetativa *vs*. la reproducción sexual es el bajo costo por hijo, dado que no se necesita invertir en estructuras y accesorios reproductivos (flores, producción de néctar, polen, etc.) (Eguiarte *et al.*, 1999). Sin embargo, una gran desventaja de la propagación clonal en contraparte con la reproducción sexual es la ausencia de recombinación, así como su limitada capacidad de dispersión, lo cual puede tener serias implicaciones genéticas (Hartl y Clark, 1989; McLellan *et al.*, 1997) y reproductivas (Handel, 1985; Charpentier *et al.*, 2000). Además, se ha sugerido que especies clonales con tamaño poblacional finito pueden ser propensas ha acumular mutaciones desfavorables promoviendo que la adecuación y tamaño poblacional de la especie disminuyan rápidamente (Muller, 1964; Lynch y Gabriel, 1990; Eguiarte et al., 1992; Gabriel *et al*., 1993).

La capacidad de propagación clonal es un fenómeno común entre las plantas (Harper, 1977; Klimes *et al.* 1997) y se ha concebido como un mecanismo muy ventajoso y poco costoso de persistencia de una población, dado que permite unir alguno de los beneficios que ofrece el no reproducirse sexualmente con los de la

reproducción sexual (Eguiarte *et al.*, 1999). Tiffeny y Nikles (1985) y Klimes *et al.* (1997) sugieren que la capacidad de propagación clonal constituye una adaptación a condiciones ambientales extremas (i.e. las que se presentan en la tundra). A pesar de que la gran mayoría de especies clonales se reproducen sexualmente, los eventos de reclutamiento por vía sexual (plántulas) en las poblaciones de especies clonales son muy raros o escasos (Eriksson, 1993). Por esto, la propagación clonal es una forma de crecimiento que tiende a maximizar la adecuación de los genets ya establecidos y permite eventualmente la colonización de nuevos ambientes mediante la producción de ramets. Se define a los ramets como aquellas partes producidas clonalmente por una planta con raíz propia y que potencialmente pueden llevar una vida independiente; a su vez, un genet es el conjunto de ramets que se originan de un mismo embrión (Abrahamson, 1980; Silander, 1985; Harper, 1985). La hipótesis más aceptada con respecto al mantenimiento de la reproducción sexual en especies clonales es que ésta permite la creación y mantenimiento de una alta diversidad genética necesaria para adaptarse a cambios ambientales (Williams, 1975; Bell, 1982). Además, la reproducción sexual proporciona ciertas ventajas ecológicas con relación a la naturaleza sésil de las plantas, debido a que las semillas producidas sexualmente pueden permanecer en el tiempo (latencia) y en el espacio (dispersión a grandes distancias) (Silander, 1985; Eriksson 1997; Charpentier *et al.*, 2000).

Una gran variedad de plantas perennes combinan la reproducción sexual y la propagación vegetativa como mecanismos de regeneración de sus poblaciones (Abrahamson, 1980; Richards, 1986). En ambientes hasta cierto punto impredecibles, tales como los desiertos, la propagación vegetativa resulta ser un mecanismo de regeneración alternativo de sobrevivencia (Parker y Hamrick, 1992; Mandujano, 1995), debido a que las condiciones extremas de temperatura y lo poco predecible de los

eventos de precipitación (Polis, 1991) impiden que el reclutamiento sexual se lleve a cabo regularmente.

Se ha reportado que algunas especies de plantas suculentas de zonas áridas o semiáridas no presentan reproducción sexual, y/o que el reclutamiento por esta vía es esporádico (Mandujano, 1995). Para que la reproducción sexual tenga lugar en ambientes desérticos, las plantas deben superar muchos obstáculos ambientales que pueden afectar la disponibilidad de recursos y el número de estructuras reproductivas (Johnson, 1992; Bowers, 1997). Por este motivo, los reclutamientos por vía sexual (por semilla) suelen ser muy esporádicos en estos ambientes, aún cuando las plantas produzcan semillas viables de manera consistente (Mandujano *et al.*, 1996). Jordan y Nobel (1979), Turner (1990) y (Pierson and Turner 1998) sugieren que en ambientes áridos, el reclutamiento de plántulas está restringido a "ventanas de oportunidad o pulsos" (ver Jeliski y Ckeliak, 1992), asociadas a eventos climáticos particulares (*e.g*. fuertes precipitaciones) que ocurren con poca frecuencia. Sin embargo, diversas especies de plantas suculentas pueden superar estas dificultades a través de la propagación vegetativa o clonal, lo que permite a las especies combinar ambos modos de reproducción (sexual y vegetativa) y asegurar su permanencia (Parker y Hamrick, 1992; Mandujano, 1995; Clark, 2000).

La propagación clonal permite que un solo genet pueda ocupar desde un área de unos centímetros hasta cientos de metros (Cook, 1985). Debido a esto, un genet puede colonizar grandes áreas y formar una población compuesta de un solo genotipo. Ejemplos de esto se han documentado en plantas arbóreas (Cheliak y Pitel, 1984) y plantas acuáticas (Klimes *et al.*, 1997). El hecho de que las unidades estructurales de un organismo clonal sean capaces de producir un nuevo individuo plantea el problema de reconocer la identidad del individuo (Janzen, 1977; Cheliak y Pitel, 1984; Bayer 1990;

Mendoza, 1994; Montalvo *et al.* 1997). Decidir si los tallos que se observan en el campo pertenecen a uno o varios genets es una tarea difícil, sobre todo cuando las conexiones se descomponen y desaparecen, quedando las partes aéreas como tallos independientes (Mendoza, 1994). Cuando esto ocurre es casi imposible contar el número de genets en una muestra poblacional, a menos que exista algún tipo de polimorfismo genético que permita distinguir diferentes genets (Harper, 1978), tales como los marcadores moleculares (i.e isoenzimas, RAPD´s y microsatélites (SSR).

Ahora bien, la capacidad de un organismo clonal de colonizar grandes áreas puede tener serias implicaciones en la función y mantenimiento de la reproducción sexual en sus poblaciones, debido a que el crecimiento clonal puede interferir con los patrones de polinización y el sistema reproductivo (Handel, 1985; Charpentier *et al.*, 2000, Eckert, 2000; Charpentier, 2002). Por ejemplo, dentro de una población clonal un mismo genotipo puede ocupar una gran extensión de terreno gracias a la propagación vegetativa. En este caso podría incrementarse la probabilidad de geitonogamia (i.e. polinización entre dos flores de un mismo individuo genético o genet) (Handel, 1985; Back *et al.*, 1996; Charpentier *et al.*, 2000, Eckert, 2000; Charpentier 2002) y con ello generar altos niveles de endogamia, producto de autopolinización o cruzas entre parientes (Trame *et al.* 1995; Charpentier, 2002; Frankham *et al.*, 2002). El crecimiento clonal en una especie auto-incompatible, además de tener un costo en términos de incrementar los niveles de endogamia, puede generar un gran desperdicio de polen producto de cruzas entre individuos genéticamente similares pero separados espacialmente fisicamente (entrecruzamiento óptimo, Waser y Price, 1989, 1991; Trame *et al.* 1995) o producto de cruzas entre individuos adaptados a diferentes condiciones (depresión por exogamia, Waser y Price, 1994), lo cual conduce al igual que altos niveles de geitonogamia a un decremento en la adecuación de los individuos.

En biología de la conservación el conocimiento de los mecanismos de reproducción, de la dinámica poblacional y de la estructura genética de una especie, es de vital importancia e interés práctico, ya que frecuentemente tiene relevancia en el planteamiento de estrategias de manejo y conservación de las especies. Una población que contenga una gran cantidad de genets diferentes puede, por ejemplo, ser tratada en forma diferente a una compuesta de muchos ramets de un mismo genet. En este sentido, la estrategia para poblaciones compuestas de una gran cantidad de genets, sería conservar solo una población, mientras que para poblaciones compuestas de uno o pocos genets la estrategia sería conservar varias poblaciones, debido a que estás poblaciones estarían sujetas a mayores presiones de índole genético, reproductivo y demográfico, por ejemplo la pérdida de un genet (producto de enfermedades o factores antropogénicos) podría significar que la especie presente una reducción substancial o total de su variación genética.

Stenocereus eruca es una cactácea clonal que presenta una distribución restringida a las Planicies de Magdalena, en Baja California Sur, en el Desierto Sonorense (Gibson, 1989; Turner *et al.*, 1995). Se trata de una especie biológicamente interesante, ya que es una de las dos únicas cactáceas del grupo columnar que presentan hábito rastrero (S. *eruca* distribuida en Norteamérica y *Trichocereus thelegonus* en América del Sur); además, se le considera como la especie con habito clonal más marcado dentro de la familia Cactaceae (Gibson y Nobel 1986). En este sentido, Gibson (1989) y Turner *et al.* (1995) han sugerido que esta especie se mantiene predominantemente por propagación vegetativa, debido a que no se han observado plántulas en el campo. *Stenocereus eruca* se encuentra incluida dentro de la Norma Oficial Mexicana con estatus de amenazada (NOM-059-ECOL-2001), debido a que el hábitat que ocupa está en riesgo inminente por el avance de las actividades agrícolas de

la región, así como la colecta ilegal por parte de coleccionistas y vendedores de cactus (Cancino *et al*., 1995).

Los antecedentes anteriores hacen de *S. eruca* un sistema biológico muy interesante para abordar el estudio de aspectos relacionados con su estructura clonal, su comportamiento demográfico y su biología reproductiva. A pesar del gran interés biológico que ha habido en torno al estudio de plantas clonales en las últimas dos décadas, nuestro conocimiento actual sobre la interacción entre la estructura clonal y el comportamiento demográfico, en asociación con la biología reproductiva de las plantas es muy escaso y en muchos casos ha llevado a resultados conflictivos. En este trabajo se abordan aspectos genéticos, demográficos y reproductivos con la finalidad de tener una comprensión integral del comportamiento poblacional de esta especie.

Para la realización de este trabajo se plantearon los siguientes objetivos:

- Evaluar la estructura espacial de genets con la finalidad de estimar la importancia relativa del reclutamiento vía sexual *vs*. clonal utilizando como marcador a los RAPD´s (Random Amplification Polimorphic DNA).
- Evaluar las consecuencias demográficas de la reproducción sexual y la propagación clonal, así como la importancia relativa de cada estrategia reproductiva.
- Analizar el efecto de la variación espacio-temporal en la abundancia de polinizadores sobre la fecundidad de esta especie e inferir el papel de la clonalidad en su sistema reproductivo.

La integración de diversas herramientas en esta investigación permitirá aportar evidencias sobre la importancia relativa de dos procesos demográficos básicos: el reclutamiento vía sexual *vs*. la propagación clonal, usando una combinación de datos genéticos, demográficos y reproductivos. La integración de datos genéticos y

demográficos permitirá evaluar el comportamiento demográfico a nivel de genets y ramets. La integración del análisis genético con el estudio del sistema reproductivo permitirá evaluar los efectos de la estructura clonal sobre la reproducción y, por último la integración del análisis demográfico con el del sistema reproductivo permitirá conocer las causas de la baja fecundidad y bajos reclutamientos que se han observado en las poblaciones de *S. eruca*. La integración de toda esta información aportará, sin duda, bases fundamentales para la conservación de esta especie amenazada.

De acuerdo a lo anterior, esta tesis se realizó siguiendo tres líneas de investigación principales. Cada línea de investigación constituye un capitulo, como se describe a continuación:

CAPITULO 1.- DIVERSIDAD CLONAL Y ESTRUCTURA GENETICA. El objetivo de esta sección es evaluar los niveles de diversidad genotípica, lo que permitirá estimar la importancia relativa de la reproducción sexual y la propagación vegetativa, utilizando como marcador molecular a los RAPD´s. Asimismo, dentro de este capitulo se describe la distribución espacial de los clones en una población de esta especie.

CAPITULO 2.- DEMOGRAFIA DE GENETS Y RAMETS. El objetivo de este capítulo es evaluar el comportamiento demográfico de genets y ramets con la finalidad de estimar la importancia relativa de la reproducción sexual *vs*. propagación clonal desde el punto de vista de la dinámica poblacional. Asimismo, se pretende evaluar las condiciones demográficas actuales de las poblaciones de *S. eruca,* especie sujeta a condiciones severas de perturbación antropogénica, lo cual coadyuvará a plantear estrategias de conservación para esta especie. Dado que el análisis genético del capítulo anterior permitió conocer la identidad genética de cada individuo, este capítulo se abordó desde un contexto genético-demográfico, en donde la unión de ambos puntos de

vista permitirá analizar con mayor detalle la importancia relativa de la reproducción sexual y vegetativa.

CAPITULO 3.- BIOLOGIA REPRODUCTIVA. El objetivo de esta sección es explorar el efecto de la variación espacio-temporal en la abundancia de polinizadores sobre la eficiencia reproductiva y evaluar la importancia de la reproducción sexual de esta especie. En este capítulo se pretende evaluar si las bajas fecundidades de la especie son producto de: a) una limitación por polinizadores o de b) una limitación por polen debido a eventos de geitonogamia.

Capitulo I.

DIVERSIDAD CLONAL Y ESTRUCTURA GENETICA

Clonal diversity and distribution in *Stenocereus eruca*, a narrow endemic cactus of Sonoran desert

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Stenocereus eruca (Cactaceae) is a prostrate cactus, endemic to the Sonoran desert that exhibits one of the most extreme cases of clonal propagation in the cactus family. We examine clonal diversity and distribution in this species at two spatial scales: i) at the population level, in four populations along its distribution range; and ii) at a small scale, within a 600 m^2 plot at Estero Salinas. Our objectives were to evaluate the relative importance of sexual vs. clonal recruitment, using random amplified polymorphic DNA (RAPD) markers. Six primers produced a total of 75 scorable markers, 57 of which (77%) were polymorphic. Contrary to expectations based on field studies, clonal diversity was relatively high across the distribution range, suggesting that sexual recruitment is an important regeneration mechanism. Thus, the proportion of distinguishable genotypes and genotypic diversity (G/N = 0.83, D = 0.987) were greater than values detected in other clonal cacti, suggesting that clonal propagation is not the major mechanism of regeneration. Autocorrelation analyses revealed a spatial genetic structure that may be the result of restricted gene flow (via pollen or seeds) and clonal propagation. High levels of genetic variation were detected at both the population and the small-scale levels (Hs = 0.28±0.02, P = 70.6±6.6 and Hs = 0.36, P = 98.0, respectively). A molecular variance analysis (AMOVA) indicated that most of the variation (66.3%) was found within populations. Future studies on pollen and seed dispersal are suggested in order to understand the role of the clonal habit on the mating system of *S. eruca*.

Key words: clonal diversity; clonal propagation; RAPD; sexual reproduction; spatial genet structure; *Stenocereus eruca*.

Introduction

Many perennial plants combine sexual reproduction and clonal propagation as population regeneration mechanisms (Abrahamson, 1980; Richards, 1997). In some clonal species the relative success of sexual vs. clonal recruitment often varies geographically in response to ecological and genetic factors that limit one or the other regeneration mechanism (Eckert, 2002). The demographic balance between sexual and clonal recruitment is likely to have important consequences in the clonal diversity and the genetic structure of plant populations (Ellstrand and Roose, 1987; Eckert and Barrett, 1993). Seedling recruitment in clonal species vary along a continuum from repeated seedling recruitment, when seedlings get established on a regular basis, to initial seedling recruitment, when seedlings get established only during the initial colonization of a site (Eriksson, 1993; 1997). The pattern of seedling recruitment influences the maintenance of genotypic diversity as it is known that without new seedling input

and no selection, clonal diversity is expected to rapidly decline (Watkinson and Powell, 1993). In contrast, even low rates of seedling recruitment are sufficient to maintain relatively high levels of genotypic variation within plant populations (Soane and Watkinson, 1979; Watkinson and Powell, 1993). Thus, studies that document clonal diversity allow inferences about the relative role of sexual vs. clonal recruitment in the regeneration of plant populations.

In arid environments the population dynamics of many plant species is highly variable as a consequence of extreme temperatures and unpredictable rainfall events (Polis, 1991). In these environments seedling recruitment of succulent species is often restricted to pulses during rare climatic events of heavy rainfall (Jordan and Nobel, 1979; Turner, 1990, Pierson and Turner, 1998), even when viable seeds are consistently produced (Mandujano *et al.* 1998; Fleming *et al*. 1996). In contrast, clonal propagation is often a successful mechanism of regeneration in populations of succulent species (Parker and Hamrick, 1992). Nonetheless, our knowledge about the clonal structure and the relative importance of clonal vs. sexual recruitment in clonal cacti is poor.

Stenocereus eruca is a clonal columnar cactus of the Sonoran desert (Gibson, 1989, Turner *et al.* 1995). It is an extremely narrow endemic species, restricted to coastal areas of the Plains of Magdalena in Baja California (Gibson, 1989, Fig 1). The flowers of *S. eruca* are self-incompatible; fruit set is in general low and highly variable in space and time as a consequence of pollinator limitation associated to variation in sphingid abundance (Clark-Tapia and Molina-Freaner, in press). Seedling recruitment has seldom been observed in natural populations of *S. eruca* and thus, clonal propagation is thought to be the major regeneration mechanism (Gibson, 1989, Turner *et al.* 1995). A 3-yr demographic study of *S. eruca* suggests that the relative contribution of sexual recruitment is less important than clonal propagation to population growth rate (λ) (Clark-Tapia, et al., submitted). *S. eruca* is considered as the most extreme case of clonal propagation in the cactus family (Gibson and Nobel, 1986). Clonal propagation occurs by detachment of branches from the major shoot as the base of the branch dies and rots. Thus, in *S. eruca* the ramets (the vegetative production of modular units, sensu Harper, 1977) of a genet (all products of a zygote) tend to detach and fragment. Therefore, the genetic similarity among ramets can only be studied using genetic markers (*i.e*. isozymes or DNA based markers). A previous study on the clonal diversity of *S. eruca* using isozymes suggested that both sexual and clonal recruitment are important in the regeneration of its populations (Clark-Tapia 2000). Isozyme evidence also showed moderate levels of genetic variation (H_e = 0.158, P = 46.2), substantial deviation from Hardy-Weinberg expectations (f = 0.739), low genetic differentiation among populations (? = 0.069) and no evidence of isolation by distance (Clark-Tapia 2000). However, DNA-based markers usually have higher resolution power than isozyme markers, which allows a more precise characterization of genotypic diversity in clonal plant populations (Peakall *et al.* 1995; Ayres and Ryan, 1997). In this study we used random amplified polymorphic DNA markers (RAPD) to analyze the clonal diversity and genetic structure of the clonal columnar cactus *S. eruca* in order to: (1) evaluate the relative importance of sexual vs. clonal recruitment in four populations of *S. eruca*, (2) describe the spatial distribution of genotypes in one population to explore whether they have a random or an aggregated distribution, (3) describe the genetic structure of four populations of *S. eruca*, and (4) explore which model of seedling recruitment is consistent with the observed level of clonal diversity within populations.

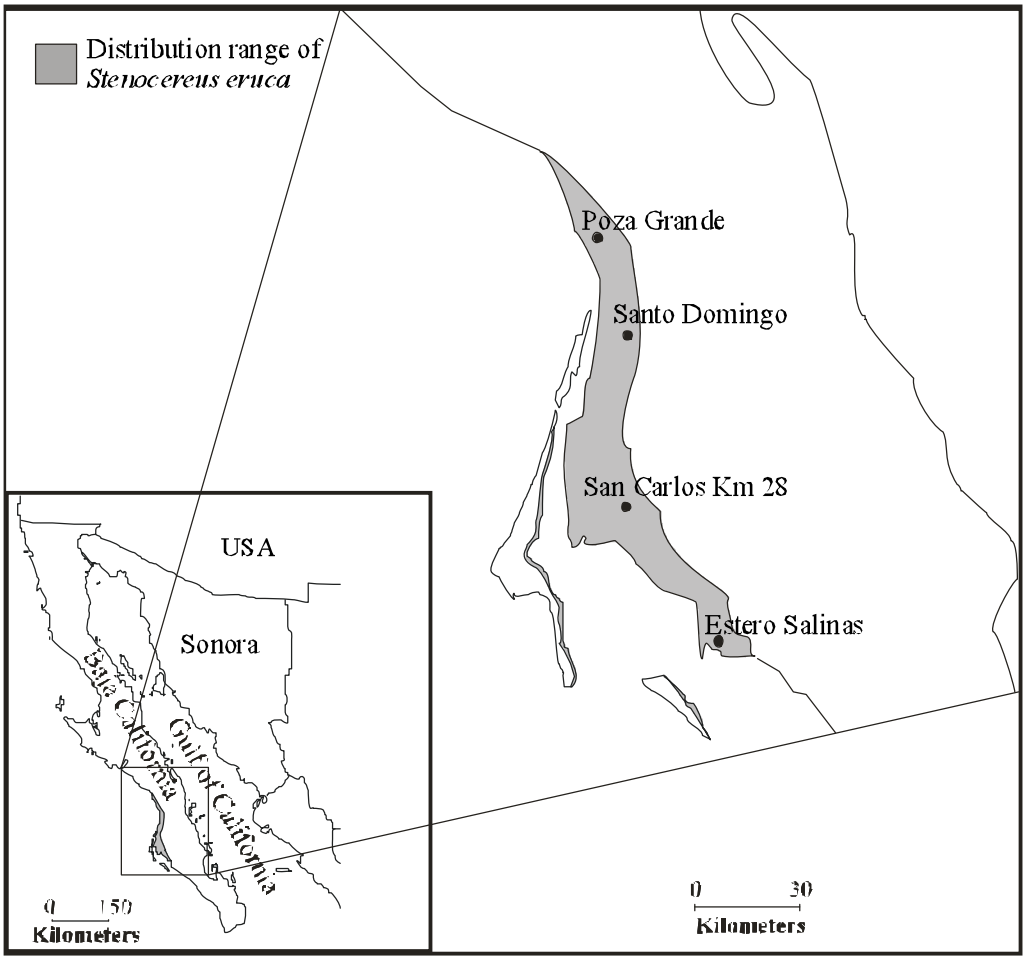

Fig. 1. Range of distribution (shaded area) of *Stenocereus eruca* in Baja California and the location of the studied populations. Modified from Turner et al., (1995).

Material and Methods

Collection of genetic material.- Given that we were interested in knowing the relative importance of sexual vs. clonal recruitment across populations, we studied four populations in the Magdalena Plains of Baja California trying to cover the entire distribution range of *S. eruca* (Fig. 1). For population-level surveys, we sampled 30 ramets along a 1200 m long transect collecting tissue samples every 30-40 m in order to cover the whole population. For assessing the spatial distribution of clones, we selected the Estero Salinas population because the population density at this site (ca.4500 ramets per hectare) was much larger than at the other sites. This population is the subject of a demographic study in which all ramets within a 10 x 60 m plot have been tagged, numbered and mapped (Clark-Tapia et al., submitted). All ramets within the plot (n = 282) were sampled and their exact locations were recorded by measuring the distances to the plot margins. Small (2-3 cm^3) samples of rib chlorenchyma were extracted from each selected ramet using a cork borer. Tissue samples were collected during November of 2001

and were stored on ice for two days and then in an ultra-cold freezer (-70 ºC) for several more days prior to DNA extraction.

RAPD´s analysis. – Total genomic DNA was extracted using a modification of the Quiagen Plant Minikit extraction method (Sánchez *et al*. submitted). Tissue from rib chlorenchyma was frozen briefly in liquid nitrogen and ground to fine powder using a mortar and pestle. Amplification reactions of the target DNA were carried out in a total volume of 23.3 µL per sample: the reaction mixture consisted of 16µL of purified water (GibcoBRL), 2µL 10 - base primer (Operon Technologies Inc.), and 1.5 U *Taq* DNA polymerase (GibcoBRL), 10 ng of template DNA and 5.07 µL of master stock (1.38 µL of purified water, 2.6 µL of buffer minus Mg (GibcoBRL) (pH = 8.4), 0.05 µL of $MgCl_2$ and 0.26 µL (0.1mM) of each dNTPs of Pharmacia). PCRs were performed in a Techgene thermal cycler programmed for one cycle of 5 min at 94ºC followed by 44 cycles of 1 min at 94ºC, 1 min at 38 ºC, 30s at 54ºC and 2 min at 72ºC. After the last cycle a final extension step of 13 min at 72ºC was carried out. Amplification products were separated electrophorectically on a 1.4% agarose gels using 0.5 X TBE running buffer at 120 V for 2.5 hours. Gels were stained with ethidium bromide and visualized under UV light, recording the image with a digital camera.

Forty primers were screened (Operon Technologies Inc. kits A and G) for reproducible amplification patterns using DNA test samples from four different populations of *S. eruca*. Six informative and reproducible primers (A01, A10, A15, G03, G16 and G18) were chosen and only strong polymorphic bands between 350 and 2036 bp were used for data analysis (Fig. 2). All RAPDs bands were scored as present (1) or absent (0) to construct the data matrix.

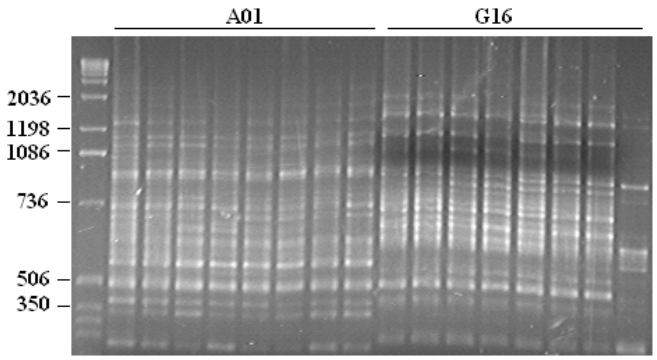

Fig. 2.- Representative amplification products of *Stenocereus eruca* samples generated by primers A01 and G16. The lane on the left contains 1kb DNA size markers. The sizes are indicated in base pairs (bp).

Data analysis

Clonal diversity.- Based on the scored RAPD banding patterns, putative genets were identified. For the estimation of clonal diversity the following parameters were calculated for the population-level survey and for the smaller scale level at Estero Salinas: (1) the proportion of distinguishable genotypes (sensu Ellstrand and Roose, 1987) was measured as *G/N*, where *G* is the number of genets and *N* is the total number of individuals sampled (ramets). (2) Simpson`s diversity index (*D*) modified for finite sample size by Pielou (1969). This index measures the probability that two ramets selected at random from a population of *N* plants will be from different multilocus genotypes, and yields a measure of multilocus genotype diversity. D ranges from 0 to 1, with 1 being the maximum diversity; this index was calculated as:

$$D = 1 - \sum \frac{n_j(n_j - 1)}{n(n - 1)}$$

where n_i is the number of individual ramets of genotype *i*, and *N* is the sample size. (3) Genotypic evenness (Fager, 1972) was measured as: $E = \frac{(Dobs - D\min)}{(D\max - D\min)}$

where $D\min = \frac{((G-1)(2N-G))}{(N)((N-1))}$, and $D\max = \frac{((G-1)(N))}{(G)((N-1))}$

and where G is the number of clones, and N is the sample size. This index ranges from 0 for a population dominated by one genotype, to 1 for a population in which all genotypes are represented by the same number of ramets.

Spatial genetic structure.- The data of band presence/absence from RAPD analysis were used to determine genetic identity and examine the spatial distribution of genets at Estero Salinas. The spatial distribution in this population was evaluated by constructing a detailed map in which the genotype of each ramet was identified and the distribution and spatial extent of genets was determined. Spatial autocorrelation analysis was performed based on the genetic similarity/dissimilarity of RAPD banding patterns found among single ramets. The spatial autocorrelation was examined using Tanimoto´s genetic distance (D_G), which is typically used with binary data such as fingerprints, and a distance measure such as the geographic distance. This analysis was performed for 10 geographical distance classes (in m, see results section) using the software Spatial Genetic Software v.1.0 c (Degen, 2001). In order to assess statistical significance and control the overall probability of mistakenly declaring D_G-values as significant, we used the 99% confidence intervals generated from 5000 permutations (cf. Degen *et al*. 2001). The spatial autocorrelation was visually examined as a correlogram, plotting D_G values, the mean of genetic distances and the 99% confidence intervals as a function of distance. If D_G (observed) is greater than the D_G (99%CI), then there is a significant autocorrelation at that distance class and therefore significant genetic structure (Degen *et al*. 2001). In addition, to analyze the spatial genetic structure within Estero Salinas as a result of clonal propagation or due to isolation by distance through limited gene flow, spatial autocorrelations were performed at both the genet and ramet levels (i.e. Reusch *et al*. 1999; Hangelbroek *et al*. 2002) using the software Spatial Genetic Software v.1.0 c (Degen, 2001). At the ramet level all samples (282) were included, while at the genet level only one ramet of each genet was taken at random from the plot.

Genetic variation.- The following estimates of variation were obtained using TFPGA software (Miller, 2000): percent polymorphic loci (P), and expected heterozygosity (H_s). Since co-dominant markers (isozymes) revealed significant deviations from Hardy-Weinberg (Clark-Tapia 2000), a molecular variance analysis (AMOVA, Excoffier et al. 1992) was used to describe population structure, where the variance was partitioned within and among populations. Pair-wise genetic distances ($?_{st}$) among the four populations and their significance levels were also obtained from AMOVA. All the analyses were performed with the AMOVA-PREP program version 1.01 (Miller, 1998) and WINAMOVA program version 1.55 (Excoffier et al. 1992, 1993). The AMOVA program generates $?_{st}$ a parameter analogous to Wrigth F_{ST} (an indicator of the degree of differentiation among population) facilitating comparison of results with other studies. $?_{st}$ was used to calculated the number of migrants per generation entering each population (Nm) using the formula $Nm = ((1/?_{st})-1)/4$ a, where $a = (n/n-1)^2$, and n is the number of subpopulations (Crow and Aoki, 1984). Isolation by distance was tested by correlating genetic and geographic distances among populations using the Mantel test (Mantel, 1967) as implemented in TFPGA (Miller, 2000).

Results

Clonal diversity.- The clonal diversity detected in the population-level survey was high. From 30 ramets examined in each population, we detected a large number of distinct genets (i.e. different RAPD banding pattern). The number of putative genets in each population ranged from 24 to 26 (Table 1). The proportion of distinguishable genets (G/N), genotypic diversity (D) and evenness (E)

were generally high and similar among populations (Table 1).

The clonal diversity at the smaller-scale level, in the plot sampled at Estero Salinas, was lower than the population-level survey (Table 1). From 282 sampled ramets, 110 different putative genets were detected. The mean proportion of distinguishable genets (*G*/*N*) within this site was 0.39. Estimates of Simpson's diversity index (*D*) and evenness (*E*) were 0.940 and 0.907, respectively (Table 1).

Table 1.- Parameters of genetic and clonal diversity in four populations of *S. eruca.* Values for the population-level survey and the small-scale level are shown separately. *N*, number of individuals sampled per population; *Hs*, expected heterozygosity, *P* (99% criteria), percentage of polymorphic loci, *Gm*, observed number of genotypes, *G*/*N*: proportion of distinguishable genotypes, *D*, genotype diversity and *E*, genotype evenness. *SD* is the standard deviation

Species/Population	N	*Hs*	*P*	*Gm*	*G/N*	*D*	*E*
Population-level survey							
A- LA POZA GRANDE	30	0.253	61.90	24	0.800	0.986	0.864
B- SANTO DOMINGO	30	0.279	71.43	25	0.833	0.986	0.743
C- SAN CARLOS	30	0.272	71.43	25	0.833	0.986	0.743
D- ESTERO SALINAS	30	0.301	77.78	26	0.867	0.991	0.791
Mean		0.277	70.64	25	0.833	0.987	0.785
SD		0.020	6.55		0.027	0.003	0.057
Small-scale							
D- ESTERO SALINAS							
Mean	282	0.359	98.0	109	0.387	0.940	0.853

Spatial distribution.- The spatial distribution of genets at Estero Salinas is shown in Fig. 3. Genets had from 2 to 41 ramets, with a mean number of 2.4 ± 5.1 ramets (± 1 SD), while 32% of the ramets had a different unique putative genet. Most of the sampled ramets of a particular genet were growing close to each other, showing a clumped distribution (Fig. 3). However, we found several genets with an extensive distribution, the largest genets (number 15 and 17) spreading over the entire plot (600 m^2). The greatest numbers of ramet pairs with identical RAPD band patterns were separated by distances between 0 and 20 m (Fig. 4). The number of identical genets decreased as distance among pairs increased (Fig. 4).

The correlogram of the 282 ramets from Estero Salinas revealed a significant positive spatial

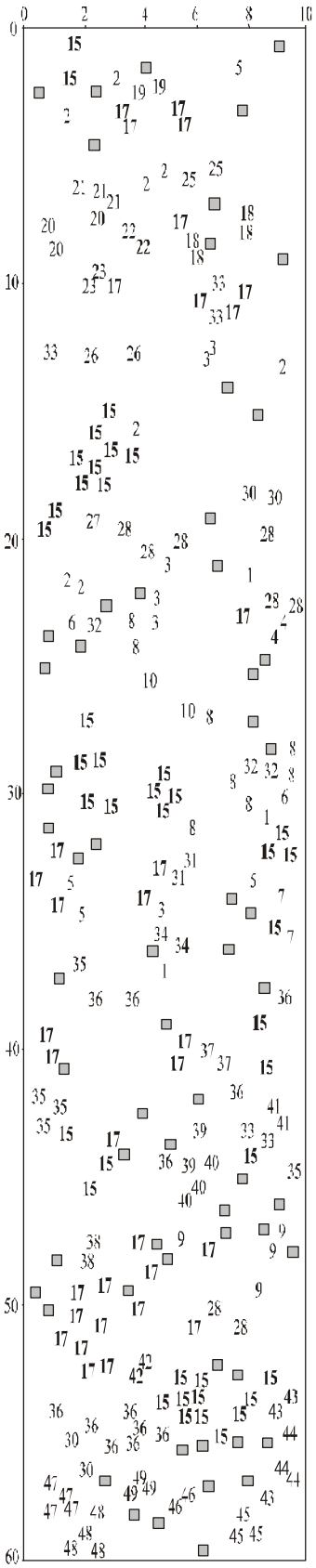

Fig. 3.- Spatial distribution of *Stenocereus eruca* genets in the Estero Salinas population, using 52 polymorphic RAPD markers. Numbers that are repeated represent ramets from the same genet. Squares without number represent unique putative genets. Distances between genets in the map are proportional to distances measured in the field. Genets represented by a large number of ramets are shown in bold numbers.

autocorrelation over distances shorter than 7 m and negative spatial autocorrelation beyond approximately 29 m (Fig. 5a). The correlogram and the 99 % confidence intervals of D_G for the 109 genets show a similar pattern (Fig. 5b). Although, correlograms show similar spatial patterns at the two levels (ramet and genet), with positive correlations at smaller spatial scales, it is important to note that the degree of relatedness (e.g. the magnitude of D_G) differed markedly between levels. The ramet level had relatively small significant values of D_G for the first two distance classes (< 11 m) than the genet level.

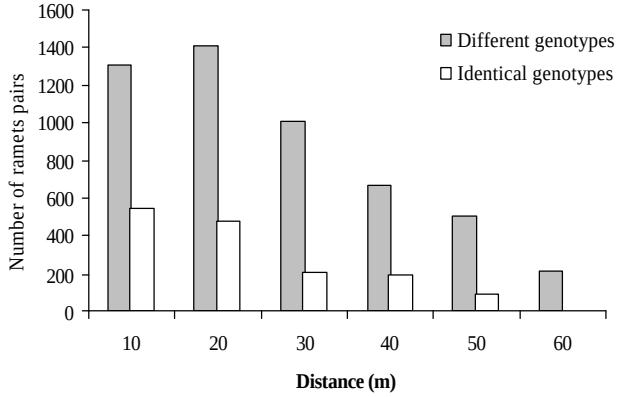

Fig. 4.- Frequency distribution of interplant distances (in meters) among ramets of *Stenocereus eruca* at Estero Salinas. Grey bars indicate ramet pairs with different genotypes (i.e. different RAPD banding patterns) and white bars indicate ramet pairs with identical genotypes.

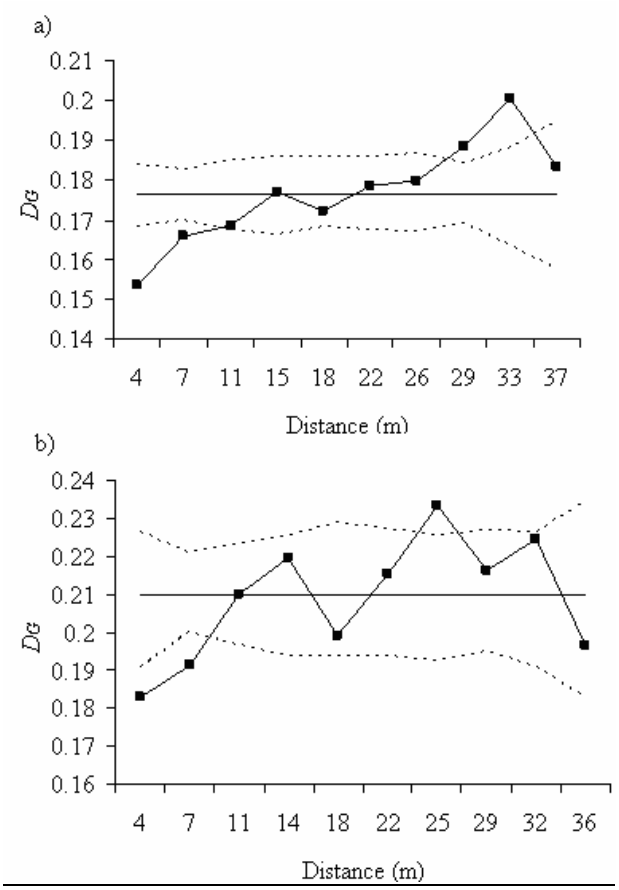

Fig. 5.- Correlograms plotting the Tanimoto distance (D_G) for all *Stenocereus eruca* individuals as a function of distance (in meters) at Estero Salinas: (a) ramet level (n = 282), and (b) genet level (n= 109). Closed squares are the observed D_G values estimated using 5000 permutations. Solid lines represent the expected values and the dashed lines represent the upper and lower 99% confidence intervals. Note that the scales of the x and y axes differ between the two graphs.

Genetic variation.- From a total of 63 bands that were analyzed, 57 (90.5%) were polymorphic at the species level. The average percentage of polymorphic loci (*P*) among populations was 71.98± 7.09 (ranging from 63.79 to 81.03%), whereas in the small-scale level at Estero Salinas it was 98%. The average genetic variation (H_s) among population was 0.279 ± 0.025 (ranging from 0.254 to 0.313), while in the plot at Estero Salinas the average H_s value was 0.359 (Table 1). Pair-wise analysis of molecular variance (AMOVA) showed highly significant ($?_{st}$ = 0.337, P < 0.002) genetic differentiation among populations of *S. eruca* (Table 2). An estimate of the average migration per generation (*Nm*) was 0.30. No evidence of isolation by distance was detected, as genetic distances and geographical distances (in Km) among populations were not significantly correlated (Mantel test; r = 0.808, P = 0.158).

Discussion

In this study we have shown that populations of *S. eruca* are composed by a number of different genets (i.e. are multiclonal) and exhibit considerable levels of clonal diversity. In general, ramets sharing the same genotype were spatially aggregated (< 20 m), while only a few genets were spread over larger distances. Significant levels of genetic variation were found within population, while substantial differentiation was observed among populations. Overall, the available evidence indicates that *S. eruca* combines sexual recruitment and clonal propagation as mechanisms of regeneration.

Contrary to expectations based on field observations (Gibson 1989, Turner *et al*. 1995), clonal diversity was relatively high across the distribution range of *S. eruca*. This is a surprising result since seedling recruitment has not been detected in the field and the results of a demographic study currently in progress suggest that clonal propagation is the most important mode of regeneration (Clark-Tapia et al., unpublished). However, the results presented in this paper are consistent with those of other studies that have found high levels of clonal diversity in plant species in which seedling recruitment has not been observed in short term studies (Jonsson *et al*. 1996; Verburg *et al*. 2000; Hangelbroek *et al.* 2002). Given that without seedling recruitment clonal diversity is expected to rapidly decline and populations become dominated by a few large genets (Watkinson and Powell 1993; Eriksson, 1993), our evidence of high clonal diversity suggest that sexual recruitment is a very important mechanism of

Table 2.- Summary of the analysis of molecular variance (AMOVA) on four populations of *Stenocereus eruca*. The significance of the $?_{st}$ test was based on 5,000 permutations.

Source	d.f.	Sum of Squares	Variance component	% Of total variance	$?_{st}$	P
Among populations	3	264.05	2.75	33.71	0.337	< 0.002
Within populations	116	628.13	5.41	66.29		

regeneration in populations of *S. eruca*. Therefore, the available evidence suggests that seedling recruitment may be restricted to "narrow windows of opportunity" (Jelinski and Cheliak, 1992; Eriksson and Fröborg 1996) when favorable conditions occur during rare climatic events of heavy rainfall. Those pulses might be rare events in time and space that are difficult to detect in the ecological time scale of demographic studies.

The parameters of genotypic diversity and evenness found in *S. eruca* were within the range of values detected for other clonal species using isozymes (Ellstrand and Roose, 1987; Widen et al.1994) and RAPDs (Hangelbroek *et al.* 2002). The estimates of population-level clonal diversity were greater than the ones detected for *Lophocereus schottii* using isozymes (Parker and Hamrick, 1992) and suggest that *S. eruca* is not really the most remarkable case of clonal propagation in the cactus family (Gibson and Nobel 1986). Our results on different levels of clonal diversity at the population level survey ($G/N = 0.83$) and in the smaller-scale analysis ($G/N = 0.39$), revealed a scaling effect associated with the sampling scheme. Clonal diversity usually shows an association with the sampling scale and the size of the sampling grid, because collecting more ramets at finer spatial scales increases the probability of genets being sampled repeatedly (Cheliak and Pitel 1984; Widén *et al.*1994).

Our data on the distribution of genotypes at Estero Salinas suggest that, in general, the ramets sharing the same genotype show a clumped distribution, while a few genets are widely distributed. Most ramet pairs with identical genotypes were located at distances of less than 20 m. This pattern is likely to be the result of growth and detachment of branches, generating a fragmented genet. The evidence on the spatial genetic structure suggests restricted gene flow (pollen or seed) due to isolation by distance, or clonal propagation owing to intermingle of ramets (Fig. 5a,b). Given that *S. eruca* is self-incompatible (Clark-Tapia and Molina-Freaner, in press), pollen movement within distances less than 20 m is likely to reduce fruit and seed set (Handel, 1985; Trame et al., 1995; Charpentier et al., 2000). Future studies should address the spatial scale of pollen and seed dispersal in order to evaluate the role of the clonal habit on the mating system of *S. eruca.*

The differentiation and the estimated migration among populations ($?_{st} = 0.337$, $Nm = 0.30$) suggest restricted gene flow. Estimates of genetic differentiation observed for *S. eruca* fall within the range that has been observed for columnar cacti using isozymes (Hamrick *et al*. 2002), and are similar to those reported for RAPDs in other plant species (Nybom and Bartish, 2000; Navarro *et al*. 2003). If sexual recruitment is restricted to narrow windows of opportunity, seed-mediated gene flow among populations of *S. eruca* may be episodic and associated with rare events of heavy rainfall. Given the high temporal variation in the abundance of the major pollinators of *S. eruca* (Clark-Tapia and Molina-Freaner,

in press), pollen-mediated gene flow may also be episodic, associated with years of high sphingid activity. Thus, the considerable differentiation observed among populations could be due to the episodic and variable reproductive schedule of *S. eruca*.

Stenocereus eruca is considered as threatened under the Mexican legislation (SEDESOL, 2001), and field studies have identified habitat destruction due to agricultural development and illegal collection as major threats (Cancino et al., 1995). Our evidence on isozymes (Clark-Tapia, 2000) and RAPDs have revealed that populations possess significant levels of genetic variation without a clear geographical pattern (Table 1). Our field observations have detected evidence of increasing human disturbance that it is likely to increase fragmentation and the vulnerability of *S. eruca* to extinction (Clark-Tapia, unpubl. data). Given that human disturbance is likely to increase in the Plains of Magdalena, we suggest that at least one population should be permanently protected. Estero Salinas is probably the best option for setting a reserve as it is far from human settlements, it is the only population where we have regularly observed sexual reproduction (Clark-Tapia and Molina-Freaner, in press), it is demographically stable and ramet density is high (Clark-Tapia et al., unpubl. data). Therefore, we suggest that Estero Salinas should be protected

In summary, our data provide evidence that sexual recruitment is important in the regeneration of populations of *S. eruca.* In order to make further progress in understanding the spatial genetic structure of this species, we suggest that studies on pollen and seed dispersal would aid in the understanding of the role of the clonal habit on the mating system of *S. eruca*.

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Capitulo II

DEMOGRAFIA DE GENETS Y RAMETS

How important is clonal recruitment for population maintenance in rare plant species?: the case of the narrow endemic cactus, *Stenocereus eruca*, in Baja California, México.

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Abstract

Stenocereus eruca is a postrate columnar cactus whose regeneration seems to occur mainly through clonal propagation. It is a narrow endemic species of the Sonoran Desert in Baja California and currently considered as threatened under Mexican legislation. In this paper we describe the demography of ramets in four populations along its distribution range and the demography of genets in one population during a 3yr-study period in order to evaluate its conservation status. We also analyze the relative contribution of sexual reproduction and clonal propagation to population maintenance and provide guidelines for the formulation of conservation programs. Elasticity analyses were used to explore the relative contribution of sexual and clonal recruitment to projected population growth rate (?). During the three years of study, regeneration occurred only through clonal propagation while sexually derived seedlings were not detected within or outside the permanent plots. Our demographic data showed that the four population of *S. eruca* are in equilibrium (? ˜ 1), and elasticity analyses showed that the relative contribution to ? of clonal recruitment was larger than sexual recruitment, at least during the analyzed ecological time scale. Simulations showed that removing sexual recruitment had a minor impact on ?, but the absence of clonal propagation alone was sufficient to keep λ below unity. We propose the establishment of at least one reserve with adequate protection from human disturbance to conserve *S. eruca*.

Key word: Clonal propagation; demography; rare species; Sonoran Desert; *Stenocereus eruca*.

Introduction

The population dynamics of many clonal plants is often dominated by the birth and death of ramets produced by vegetative reproduction, because the recruitment of new seedlings of sexual origin is relatively rare or sporadic (Jordan and Nobel, 1979; Eriksson, 1989; Jelinski and Cheliak, 1992; Eriksson, 1993; McFadden, 1997; Eckert, 2002). This is especially true in arid environments, where the population dynamics of many plant species is highly variable due to extreme temperatures and unpredictable rainfall events (Polis, 1991). These conditions often lead to low rates of seedling recruitment even when viable seeds are seasonally available, whereas clonal propagation is often a successful mechanism of population regeneration (Parker and Hamrick, 1992; Mandujano et al., 1996; Mandujano et al., 2001). Thus, the relative importance of sexual and clonal recruitment varies in response to diverse environmental conditions, and their contribution to population growth rate is expected to differ (McFadden, 1991; Mandujano et al., 2001). However, our knowledge about the role of sexual *vs*. clonal recruitment in the preservation and growth of plant population in arid environments is still quite limited.

Demographic studies of clonal species that consider the dynamics of both genets and ramets are exceptionally valuable for our understanding of plant population dynamics, as inferences about the relative importance of sexual and clonal recruitment through time can be made (Harper, 1977; Hartnett and Bazazz, 1985a; Damman and Cain, 1998). The dynamics of ramets and genets may be different within a population (Eriksson 1993), yet our knowledge about the dynamics and demography of clonal plants has been restricted mainly to ramets (Sarukhán and Harper, 1973; Dickerman and Wetzel, 1985; Paciorek et al., 2000; Mandujano et al., 2001), due to the difficulty of identifying genets in the field. However, the use of molecular markers has been extremely useful in this context as they can be used to identify genets (Jonsson et al., 1996) making the study of demography at both the genet and ramet level possible (Suzuki et al., 1999).

The Cactaceae is a large plant family that is essentially restricted to the New World (Gibson and Nobel, 1986). Mexico is one of the most important centers of species diversity in the continent, possessing almost 850 species (42% of the family) of which more than 700 are endemic (Arias-Montes, 1993). However, many Mexican cacti are being threatened by habitat destruction, illegal collection and trade, and land-use change toward farming and cattle ranching (Arias-Montes, 1993). Despite the importance of this plant family to the biological diversity of Mexico, our knowledge about the population dynamics and conservation status of the vast majority of Mexican cacti is extremely poor. Demographic studies of cacti have been previously employed to evaluate the current population status and to detect vulnerable stages in their populations (Contreras and Valverde, 2002; Esparza-Olguín et al., 2002; Rae and Ebert, 2002), as well as other aspects of their population biology

(Steenbergh and Lowe, 1983; Schmalzel et al., 1995; Zavala-Hurtado and Díaz-Solis, 1995, Pierson and Turner, 1998; Godínez et al., 1999; Mandujano et al., 2001). Additionally, the development of prospective analysis (*i.e.* elasticity) and numerical simulations has opened the possibility of addressing important problems on population biology and conservation issues (Caswell, 2000; Caswell, 2001). In particular, elasticity values -that compare the relative contribution of different demographic process (i.e. fecundity or clonal propagation) to ?-, have been used across taxa to evaluate which demographic process is proportionally more relevant to overall population growth (Silvertown et al., 1993) and is a valuable tool for conservation and management as it helps to determine the most vulnerable phases of a species' life cycle (de Kroon et al., 1986; Silvertown et al., 1996).

Stenocereus eruca is a clonal columnar cactus endemic to the Sonoran desert (Gibson, 1989; Turner et al., 1995). The flowers of *S. eruca* are self-incompatible; fruit set is low and highly variable in space and time as a consequence of pollinator limitation (Clark-Tapia and Molina-Freaner, in press). Clonal propagation occurs in this case by detachment of branches from the major shoot as the base of the branch dies and rots. Thus, in *S. eruca*, the ramets (the vegetative production of modular units, sensu Harper, 1977) of a genet (all products of a zygote) tend to detach and fragment. Field records have been unable to detect establishment of seedlings of sexual origin, and it is thought that population regeneration occurs mainly through clonal propagation (Gibson, 1989, Turner et al., 1995). However, a previous study of the clonal diversity of *S. eruca* populations using isozymes suggested that both sexual and clonal recruitment are important mechanisms of population regeneration (Clark-Tapia 2000). Due to the fact that seedlings are extremely rare in natural conditions, the evidence of clonal diversity suggests that seedling recruitment is restricted to "narrow windows of opportunity" (Jelinski and Cheliak, 1992; Eriksson and Fröborg, 1996) during rare events of favorable conditions (*e.g*. heavy rainfall in the area). Therefore, detailed and long-term demographic studies are necessary in order to understand the regeneration mechanisms and the relative role of sexual and clonal recruitment in the maintenance and growth of *S. eruca* populations.

Stenocereus eruca is also an extremely narrow endemic species, restricted to coastal areas of the Plains of Magdalena in Baja California (Gibson, 1989; Fig. 1) and is listed as a threatened species by the Mexican legislation (NOM-059-ECOL-2001). Major threats to the persistence of *S. eruca* populations include habitat loss associated with land-use change towards farming and illegal collection (Cancino et al., 1995). In consequence, it is critical to understand the population dynamics and its current status in order to design conservation strategies. For instance, analyzing how anthropogenic disturbance and restricted seedling recruitment affect the population growth rate is of paramount importance in order to develop appropriate management plans.

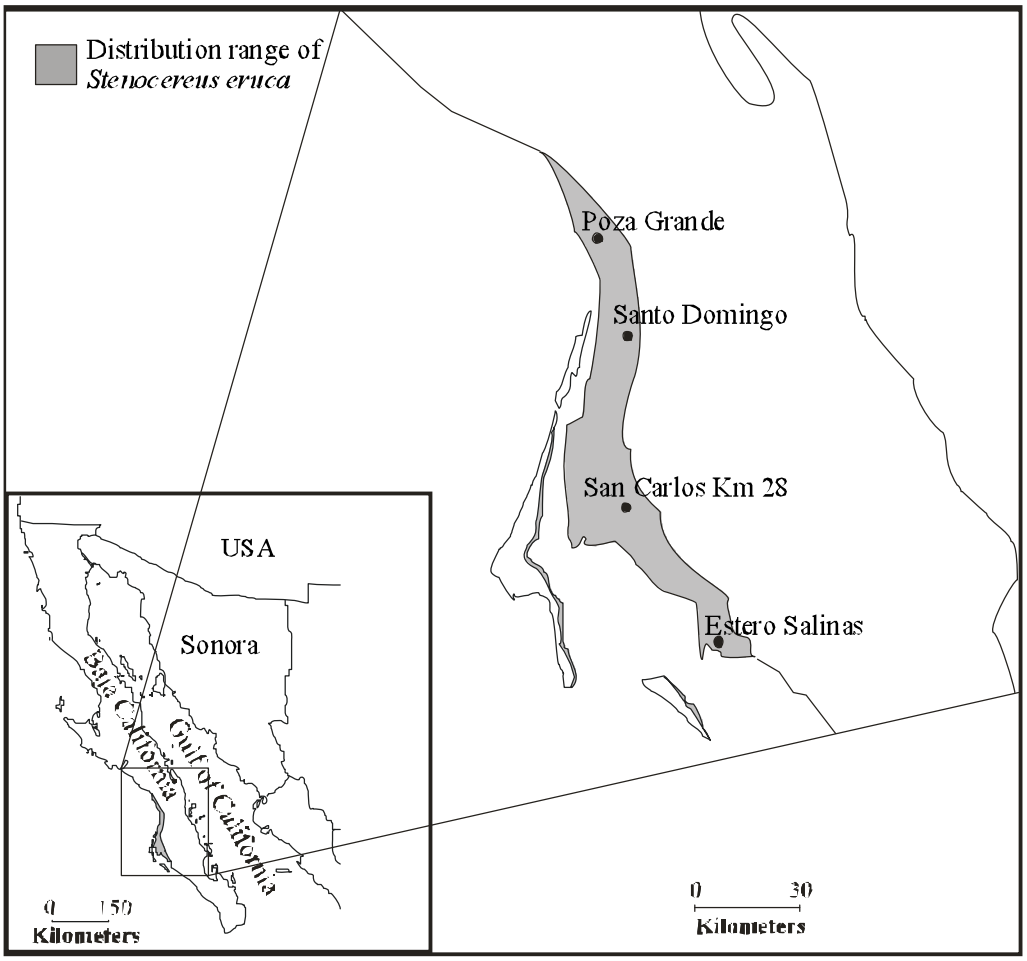


Fig. 1. Range of distribution (shaded area) of *Stenocereus eruca* in Baja California and the location of the studied populations. Modified from Turner et al., (1995).

In this study, we used demographic analysis to explore the relative importance of sexual and clonal recruitment for population growth rate in *Stenocereus eruca*. To this end, we describe the demography of ramets in four populations along its distribution range and the demography of genets (identified with molecular markers) at Estero Salinas and used elasticity analyses to evaluate the relative contribution of life history traits to projected finite population growth rate. Finally, all demographic evidence and our field observations were used to recommend conservation strategies.

Material and Methods

Study area.- This study was conducted in the Plains of Magdalena, in the Sonoran desert of Baja California Sur (B.C.S.), Mexico (Fig. 1). The Plains of Magdalena have an arid climate with mean annual rainfall of around 100 mm, and nearly 80% of the total rainfall occurring between July and September (1988-2001 data from Comisión Nacional del Agua, La Paz, B.C.S.). *Stenocereus eruca* has a patchy distribution in the area with densities of 300 to 4700 ramets/hectare.

Studied populations.- In August 1999 we selected four populations of *S. eruca* along its geographical range in the Plains of Magdalena (Fig. 1). In each population a permanent plot of 600 m^2 (10 x 60 m) was established in order to record at least 250 individual plants. All individuals inside the plots were identified with a numbered metal tag. The length of the major stem of each plant was measured with a flexible pocket tape. When a ramet had several branches, the length of each branch was measured and then added up to estimate total stem length.

Demographic parameters.- All plants within the plots were recorded and measured again in August of 2000, 2001 and 2002. In each census the following data were recorded: (1) length increase, (2) mortality, (3) seedling recruitment, and (4) detachment or fragmentation events (clonal propagation). During the fruiting season (from September to November) of 1999, 2000, 2001, and 2002 the number of fruits produced per plant was also recorded. Mature fruits outside the plots were collected every year (n ˜ 25) in order to estimate the mean number of seeds per fruit, which was used to calculate plant fecundity.

Seedling recruitment.- Each year we searched for new seedlings of sexual origin within plots and in the area surrounding each plot in every population of *S. eruca* during and after the rainy season. However, no sexual recruitment have been observed in any population. Therefore, field experiments of seed germination and seedling survival were carried out to determine the probability of recruitment. Field experiments were performed at the Estero Salinas population, because this was the only population where seeds were systematically available during the study period. To evaluate seed germination, seeds were sown in the field during the summer rainy seasons of August 2000 and August 2001, using 10 x 10 cm experimental plots. Each experimental plot contained 100 seeds, and 24 replicates were placed on the ground of which 12 were placed in open spaces and 12 beneath the cover of shrubs acting as nurse plants. To estimate the probability of seedling survival in the field, we germinated seeds in a greenhouse and seedlings were transplanted to the field in August 2001, after six months of growth (watered every 2-3 weeks) in the greenhouse. We used experimental plots containing 150 seedlings in six replicates (three plots in open spaces and three under the cover of shrubs) and watered after transplant. Seed germination and seedling survival were scored during two months, following their status until all seeds or seedlings died or disappeared. Additionally, a seed germination and seedling survival experiment under controlled condition was conducted at the Institute of Ecology (UNAM) in Mexico City during the years of 1999, 2000, and 2001, using batches of 10,000, 5,000 and 8,000 seeds, respectively. Seeds of *S. eruca* were placed in petri dishes with an agar substrate (2%) in germination chambers at 25°C. Seed germination was scored every day; two weeks after germination seedlings were transplanted to individual trays filled with homogenized soil from the study site and kept in the greenhouse. The trays

were watered every two weeks and seedling survivorship was recorded every month for one year to estimate the transition probability under controlled conditions.

Genet determination.- RAPDs (Random Amplified Polymorphic DNA) were used to identify genets and ramets within the plot at Estero Salinas due to difficulty of identifying individual genotypes in the field. Estero Salinas was selected because it had the smallest degree of human disturbance and the regularity of sexual reproduction events of this population. Each plant within the plot was mapped in a x-y coordinate for later relocation. All ramets inside the plot (n = 282) were sampled and small (2-3 cm^3) samples of rib chlorenchyma were extracted using a cork borer and stored at –80 C for later analysis. DNA was extracted using a modification of the Quiagen Plant Minikit extraction method (Sánchez et al., submitted) and six informative and reproducible primers (A01, A10, A15, G03, G16 and G18) were used for RAPD analysis. The separation and scoring of amplification products was done on 1.4% Agarose gels using 0.5 X TBE (R. Clark-Tapia, unpublished data).

Data Analyses

Matrix analysis and construction.- Once identified, genets and ramets were grouped in seven size classes according to total stem length (Table 1). Total stem length for each genet was obtained as the sum of individual length of each ramet (that shared the same banding pattern), in such a way that the selection of each size category was conditioned to the number of ramets and to stem length of each ramet. To categorize ramets we only included the total stem length of each individual in the populations. Based on measurements of annual ramet growth and the spatial expansion of clones the age of genets of *S. eruca* were estimated according to Steinger et al. (1996). Fecundity was estimated by calculating the mean number of seeds produced per size class, multiplied by germination probability. Additionally, these values were multiplied by the probability that a seedling survives for one year. Thus, no seedling survival have been detected in the field, the transition probabilities from seed to seedling was calculated using greenhouse experiments during 3-yr studied periods (1999-2000, 2000-2001 and 2001-2002). The seed class was not included in the demographic model because there is no persistent seed bank (*i.e.* seeds do not remain in the soil for more than one year.) and field observations suggest that seeds are consumed by rodents and lizards and are rapidly depleted from the population (R. Clark-Tapia, Pers. obs.).

Table 1. Size classes used in the projection matrices for genet and ramet level of *Stenocereus eruca*.

	Total length (cm)	
Stage classes	Ramet level	Genet level
1.- Seedling	0-3	0-3
2.- Nonreproductive	3.1-30	3.1-60
3.- Reproductive I	30.1-60	60.1-120
4.- Reproductive II	60.1-90	120.1-180
5.- Reproductive III	90.1-120	180.1-240
6.- Reproductive IV	120.1-150	240.1-300
7.- Reproductive V	>150	>300

Notes: Reproductive status is determined by remnants of flowers or fruits.

The population models used in this study were straightforward applications of the projection Lefkovitch matrix models (Caswell, 2001),

$n_{t+1}=\mathbf{A}\,n_t$

where n denotes a column vector whose elements n_i are the number of individuals in each category at time t or $t+1$, and **A** represents a square matrix with the transition probabilities among life cycle categories from one period to the next. Demographic processes fell into the following regions within the matrix **A**: (a) fecundity values were in the first row; (b) stasis or the probability of remaining in the same category were in the main diagonal; (c) retrogression in size or clonal propagation in the upper diagonal; and (d) growth to larger stages occupy the sub diagonals (Fig. 2). We estimated ? (finite rate of population increase), **v** (left eigenvector, reproductive value) and **w** (right eigenvector, stable size distribution) for all annual matrices as well as the mean matrix.

$$A = \begin{vmatrix} S_{11} & 0 & F_{13} & F_{14} & F_{15} & F_{16} & F_{17} \\ G_{21} & S_{22}+C_{22} & C_{23}+R_{23} & C_{24}+R_{24} & C_{25}+R_{25} & C_{26}+R_{26} & C_{27}+R_{27} \\ 0 & G_{32} & S_{33}+C_{33} & C_{34}+R_{34} & C_{35}+R_{35} & C_{36}+R_{36} & C_{37}+R_{37} \\ 0 & 0 & G_{43} & S_{44}+C_{44} & C_{45}+R_{45} & C_{46}+R_{46} & C_{47}+R_{47} \\ 0 & 0 & 0 & G_{54} & S_{55}+C_{55} & C_{56}+R_{56} & C_{57}+R_{57} \\ 0 & 0 & 0 & 0 & G_{65} & S_{66}+C_{66} & C_{67}+R_{67} \\ 0 & 0 & 0 & 0 & 0 & G_{76} & S_{77}+C_{77} \end{vmatrix}$$

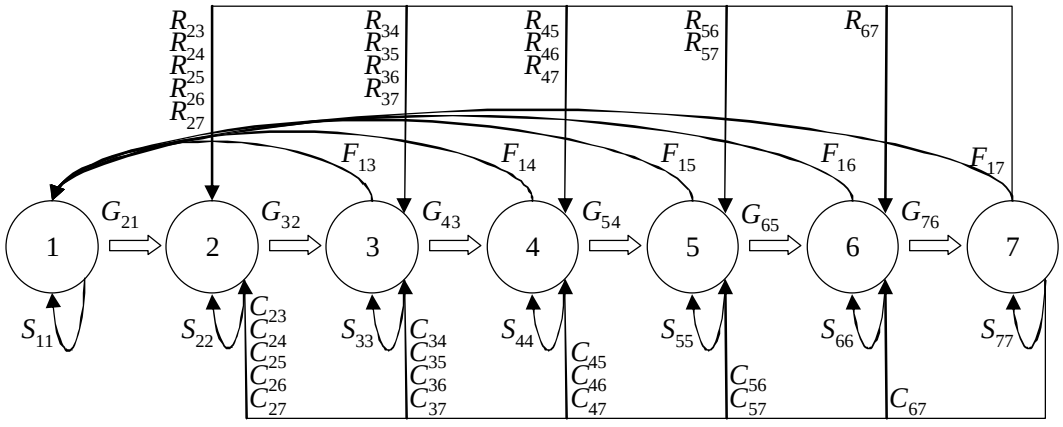


Fig. 2. Life cycle diagram and the projection matrix model corresponding to *Stenocereus eruca*. The elements inside the matrix A have the following regions: fecundity (*F*, in the first row); stasis (*S*, in the main diagonal); retrogression in size or clonal propagation (*R* and *C*, respectively in the upper diagonal); and finally growth to later stages (*G*, in the sub diagonals). See Table 1 to identify the corresponding nodes.

Annual matrices (1999-2000, 2000-2001 and 2001-2002) and a mean matrix were built for genets (only for Estero Salinas) and ramets (all four sites, see appendix). The transition probabilities among classes was calculated by the relative frequencies of each estimated transition from one year to the next or by going backwards from a group to another of smaller size (retrogression or decrease in size). Whenever the value of stasis was equal to 1.00 (no mortality occurred in individuals of the last size classes), the coefficients a_{77} for genets ($0<a_{77}>1$) and a_{77} for ramets ($0<a_{77}>1$) were calculated in a way that $a_{77}^{x} < 0.00005$, where x = the estimated time of permanence in such size classes.

Elasticity analysis

Elasticity and sensitivity analyses of projection matrices are prospective analyses, since they quantify the expected degree of perturbation to population growth given a specific change in one (or more) elements of the matrix (Horvitz et al., 1997; Caswell, 2000; Cooch et al., 2001).

Based on sensitivity values ($S_{ij} = v_i w_j / vw$), we calculated the corresponding elasticity matrices ($e_{ij} = a_{ij}/ ?)(S_{ij}$) (de Kroon et al., 1986; Caswell, 2000) for the 3yr-studied period (1999-2000, 2000-01, and 2001-02). The elasticity evaluated the relative contribution of matrix entries (a_{ij}) to the population finite rate of increase (?) and is scaled so that the sum of all values equals unity (Caswell, 2001).

Simulations excluding clonality and fecundity

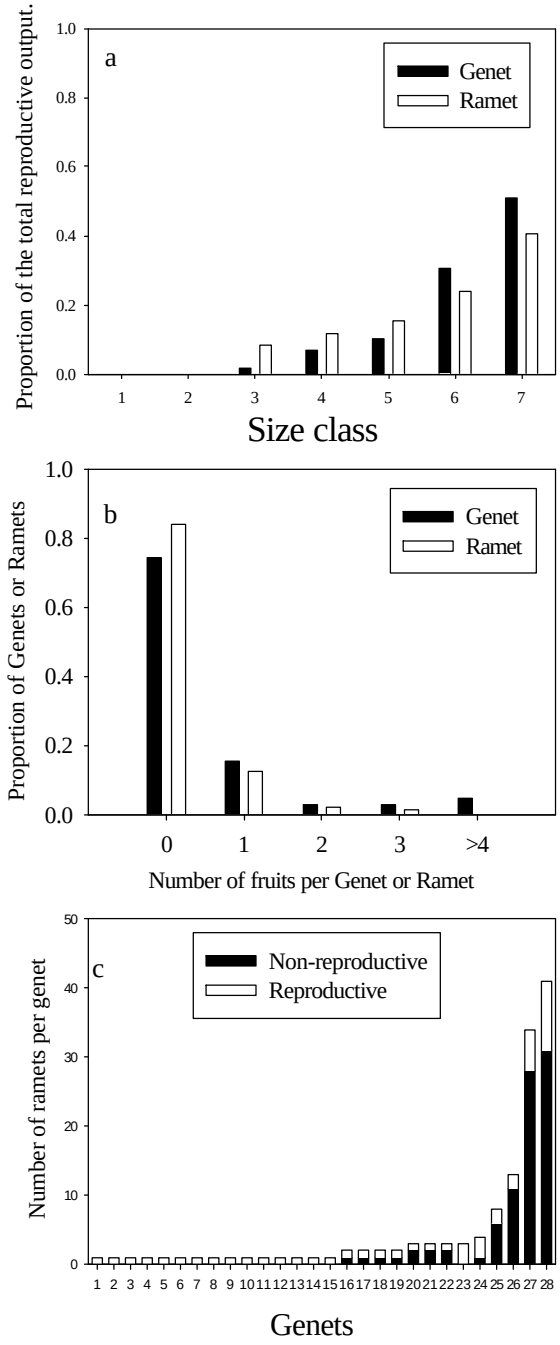
The contribution of sexual reproduction or clonal propagation to the finite rate of population increase (?) was evaluated by means of simulations. Clonal propagation or fecundity values were excluded from the ramet matrices for the 3-yr study period. To evaluate the importance of sexual reproduction the corresponding values of clonal propagation were eliminated, and to assess the importance of clonal propagation, the entries in the matrices associated to sexual reproduction were eliminated. All matrix models and elasticity analyses were carried out using MATLAB, version 5.2.0 (The MathWorks, Natick, Massachusetts, USA) and 95% confidence intervals for ? values were calculated through Monte Carlo simulations as proposed by Alvarez-Buylla and Slatkin (1993).

Results

Sexual and clonal recruitment.

Stenocereus eruca produced on average over all populations (mean over the 3yr-studied period ± 1 SD) from 0.003 ± 0.490 to 0.130 ± 0.490 fruits per ramet, and fruits produced from 211 ± 87 to 336 ± 186 seeds. Reproductive individuals (at the ramet and genet level) were found from size-class 3 to 7 (Table 1) and the highest fecundity values were detected among individuals in size classes 6 and 7 (Fig. 3a). Even though the genet analysis showed a relatively higher fecundity (based on fruit production) than the ramet analysis, no significant differences were detected between the two levels ($?^2$=0.07, P=0.89, Fig. 3b). In addition, only a small percentage of reproductive individuals were found at the ramet level (16%), whilst the genet level showed a higher percentage of reproductive individuals (26%), the largest genets showing higher reproduction (Fig. 3c).

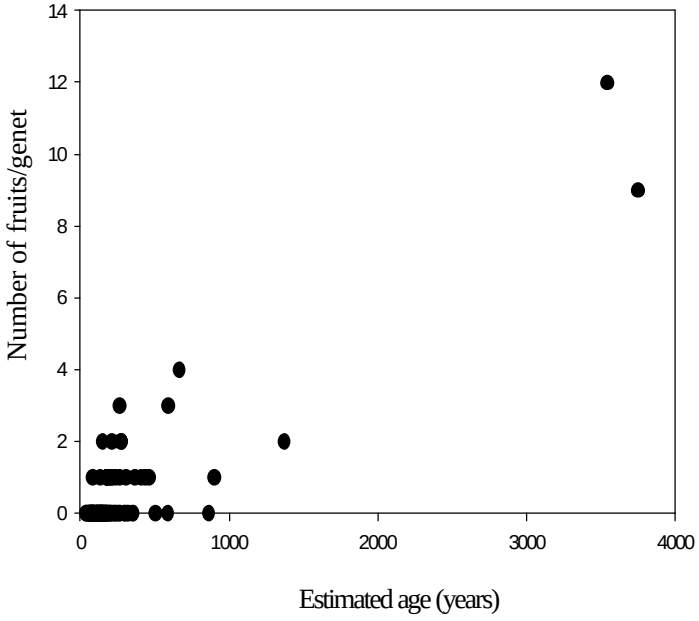
Fig. 3.- Pattern of sexual fertility among genets and ramets of *Stenocereus eruca* at Estero Salinas. a) Proportion of genets or ramets among size classes at Estero Salinas (N_{Genet}=109 and N_{ramet}=282). b) Proportion of genets or ramets among fertility classes (0, 1, 2, 3 or >4 fruits) at Estero Salinas. No significant differences were detected among levels (genet vs. ramet) when compared using a Chi square test ($?^2 = 0.07$, P = 0.89). c) Distribution of reproductive and non-reproductive ramets among fertile genets. Only 28 genets from 109 detected were reproductive.



Although seeds were produced every year, seedling recruitment was not observed during the study period within the permanent plots nor in the area surrounding the plots in each population. No seedling established in the seed germination experiments performed in the field due to seed removal by animals and seedling mortality. Similarly, seedling transplant experiments showed that no seedling survived for more than two months, presumably due to desiccation, even when found under shade of shrubs. Since no seed or seedling survived in the field, the values of germination and seedling survival used to build the matrices were those obtained from greenhouse experiments. Under controlled conditions, germination reached values of 35% to 55%, however, the probability of seedling survival after one year was extremely low (average 0.002) even under the controlled conditions of a greenhouse.

In contrast, clonal propagation reached a rate of 1.88 ± 1.33 ramets/year and varied from 1.1 ± 0.22 to 2.57 ± 2.07 among years and populations, respectively. Ramet production showed temporal variation as significant differences were detected among years ($?^2 = 6.99$, df = 2, P<0.0302), but no regional differences were observed as populations exhibited similar rates of ramet production ($?^2 = 5.07$, df = 3, P = 0.166). Ramet mortality rate was relatively low among populations and years, with a mean death rate of 0.011 ± 0.19/year varying from 0.002 ± 0.04 to 0.012 ± 0.20 among years.

Based on the scored presence/absence of RAPD bands, putative genets and their ramets were identified and their spatial distribution within the Estero Salinas plot was recorded. DNA amplification from the 282 ramets within the plot using 52 polymorphic bands yielded a total of 109 genets. Of all ramets found in the plot, 192 belonged to fragmented genets having 2 to 41 ramets (mean number of ramets per genet ± 1 SD: 2.4 ± 5.1) while 90 ramets (32%) belonged to a single unique putative genet. No genet mortality was recorded in this population during the 3-yr study period. Annual stem elongation rates of *S. eruca* ranged between 1.04 (± 4.047) and 9.97 (± 32.69) cm and, based on the size distribution of genets at Estero Salinas, an average genet may be 223 ± 433 years old. Age and fecundity of genets was strongly positively correlated (Fig. 4; Sperman rank-correlation coefficients $r = 0.541$, $n = 109$, $P < 0.001$) suggesting that fecundity increases with age.



Population growth rate (?) and stable stage distribution (w).

Based on the 95% confidence intervals, population growth rates (?) did not exhibit significant differences between years for each population, at both the ramet and genet level (Table 2). Growth rates at the ramet level varied from 1.002 (San Carlos, 2000-2001) to 1.034 (Santo Domingo, 2001-2002), while at Estero Salinas, ? values for genets ranged from 0.965 to 1.030. In all cases, growth rates were not statistically different from unity for the different years and populations (Table 2). The observed population structure and the stable stage distribution (**w**) of each population for both the ramet and genet analyses were similar and were not statistically different (log likelihood ratio $G^2 = 0.069$, df = 6, $P = 0.999$ for ramet level and $G^2 = 0.048$, df = 6, $P = 0.999$ for the genet level).

Elasticity analysis.- The elasticity analysis showed that survival (*S*) was the most important determinant of ? in all years and for the two levels of study, followed by growth (*G*) and clonality (*C*) at the ramet level, and fecundity (*F*) at the genet level (Figure 5). Fecundity and retrogression were of lesser importance in the matrices that considered the ramet level. The relative variation in the magnitude of the different demographic processes between years is very likely attributable to temporal variation in rainfall, which influences the interaction between the demographic processes.

Fig. 4. Relationship between fecundity (number of fruits/genet) and estimated age of 109 genets of *Stenocereus eruca* at Estero Salinas.

Table 2. Mean and annual values (95% confidence intervals) of the finite rate of population increase (?) calculated for four populations of *S. eruca* at the ramet level, and for one population (Estero Salinas) at the genet level, during the three study periods.

Population	Study period			
Ramet Level	1999-2000	2000-2001	2001-2002	Mean
Estero Salinas	1.016	1.014	1.027	1.021
	(0.989-1.043)	(0.982-1.046)	(0.990-1.064)	(0.987-1.055)
San Carlos	1.010	1.002	1.031	1.020
	(0.984-1.037)	(0.974-1.029)	(0.993-1.069)	(0.986-1.054)
Santo Domingo	1.024	1.023	1.034	1.028
	(0.995-1.053)	(0.996-1.051)	(0.996-1.071)	(0.992-1.065)
Poza Grande	1.013	1.020	1.019	1.017
	(0.984-1.042)	(0.988-1.051)	(0.982-1.055)	(0.989-1.045)
Genet Level				
Estero Salinas	1.030	0.985	0.965	1.004
	(1.00-1.061)	(0.961-1.010)	(0.930-1.000)	(0.968-1.040)

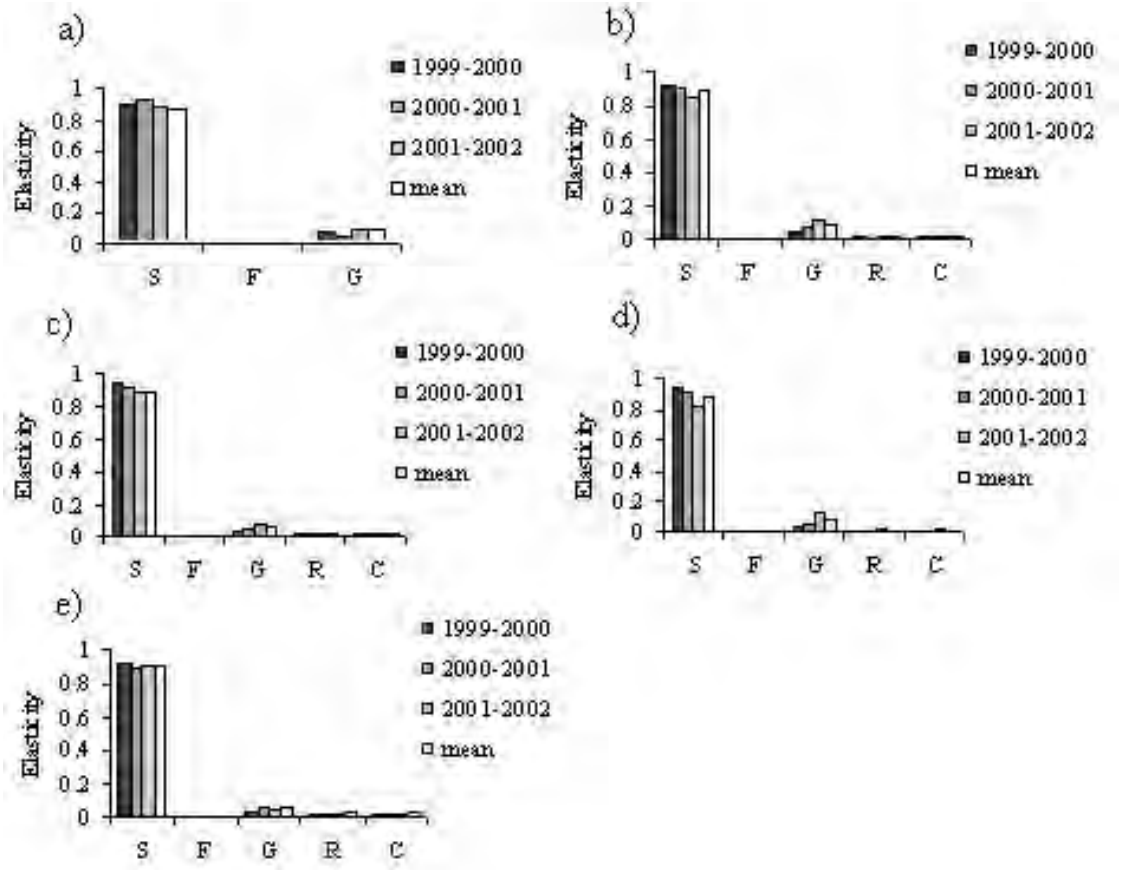


Fig. 5. Relative contribution of the different demographic processes to the value of ? from the elasticity matrices obtained for the census periods (1999-2000, 2000-2001 and 2001-2002). Elasticity values at the genet (a) and ramet level (b) at Estero Salinas; and ramet level for c) San Carlos; d) Santo Domingo and e) Poza Grande populations.

Matrix simulations without clonality or fecundity

Mean projection matrices for each population (see appendix) were used to evaluate the relative importance of sexual reproduction and clonal recruitment, as no differences were detected between populations and years. The results indicate that clonal growth is more important than sexual reproduction as population growth rate was significantly affected by the elimination of clonal propagation; therefore, the influence of fecundity at the ramet level was insignificant within populations compared to clonal propagation (Fig. 6).

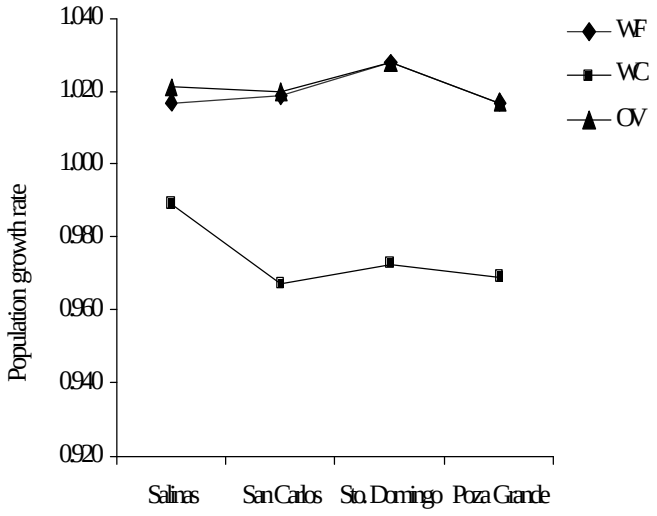


Fig. 6. Variation in simulated population growth rate (?) that resulted from populations without fecundity (◆), without clonal propagation (■) and with both fecundity and clonal growth (?).

Discussion

Our results indicate that populations of *S. eruca* exhibit stable rates of population growth that are not different from unity at either the genet and ramet level. During the three years of this study, regeneration of populations occurred only through clonal propagation, while seedlings of sexual origin were not detected within or outside the permanent plots. Elasticity analysis showed that the relative importance of clonal recruitment to ? was greater than sexual recruitment and therefore the four populations of *S. eruca* are in equilibrium mainly due to clonal growth. According to our results, clonal growth is 3-times more frequent than sexual reproduction (282 ramets/109 genets = 2.6).

Our demographic data were consistent with field observations that suggest that seedling recruitment is rare and regeneration is mainly by clonal propagation (Gibson, 1989, Turner et al., 1995), and different from what has been detected for other cacti, where seedlings have been observed to survive under field conditions (Steenbergh and Lowe, 1983; Schmalzel et al., 1995; Godínez et al., 1999; Mandujano et al., 2001; Contreras and Valverde, 2002; Esparza-Olguín et al., 2002). This pattern is similar to that observed in other clonal species where seedling recruitment is rare or restricted to “narrow windows of opportunity” even when seeds are regularly available (Jordan and Nobel, 1979; Eriksson 1993; Verburg et al., 2000; Mandujano et al., 2001; Hangelbroek et al., 2002). In contrast with demographic data, high levels of genotype diversity suggest that both sexual and clonal recruitment are important to the maintenance of populations of *S. eruca* (Clark-Tapia 2000; R. Clark-Tapia, unpublished data). This apparent contradiction between demographic and genetic evidence is likely to result from the differences in time scales that are being sampled by

ecological and genetic studies. Demographic studies usually sample brief periods of time while studies of the genotypic diversity usually capture events that occur at larger temporal scales. Thus, the available evidence suggests that on a regular basis, regeneration by sexual recruitment may be restricted to "narrow windows of opportunity" (i.e. decades or centuries) when seed escape to predation and favorable environmental conditions occur in the Plains of Magdalena.

Our results show that clonal propagation has a greater influence on population dynamics, at least at an ecological time scale. The simulations showed that removing sexual recruitment had a minor impact on the finite rate of population growth and clonal propagation was sufficient to keep λ at unity; however, eliminating clonal growth had a significant negative effect on λ. This result is consistent with other clonal species where the absence of clonal growth may increase the likelihood of extinction of populations (McFadden 1991, Mandujano et al., 2001; Rosas and Mandujano, 2002). The elasticity values of *S. eruca* are also consistent with the values reported for other clonal cacti (Mandujano et al., 2001) and show that clonal growth has a greater proportional increase in λ than sexual reproduction.

The demographic evidence of population stability agrees with other studies on long-lived plants (Piñero et al., 1984; Platt et al., 1988), and other members of the Cactaceae (Godínez et al., 1999; Mandujano et al., 2001; Contreras and Valverde, 2002; Esparza-Olguín et al., 2002; Rae and Ebert, 2002; Rosas and Mandujano, 2002). Nevertheless, some cacti with stable populations (i.e. Godínez et al., 1999; Esparza-Olguín et al., 2002; Rae and Ebert, 2002) show differences between the observed and predicted stable stage distribution, indicating that λ is not a good estimator of the current population dynamics, presumably due to high variation in both fecundity and seedling recruitment. In contrast, λ values of *S. eruca* are good estimators of the current population dynamics, as observed and expected stage distribution were equivalent. These results may suggest: (1) the absence of disturbance events in recent times, (2) a constant rate of new ramet production, or, (3) high transition probabilities to larger size classes along the life cycle (i.e. see Mandujano et al., 2001).

Demographic studies at both the genet and ramet level support the view that both levels are reciprocally influenced (Hartnett, and Bazazz, 1985b; De Steven, 1989; Mendoza, 1994; Zuidema, 2000). As other clonal plants species (De Steven, 1989; Mendoza, 1994; Zuidema, 2000), *S. eruca* promotes the perpetuation of its most successful genets through clonal propagation. Thus, a genet might achieve a larger reproductive output through clonal propagation due to a prolonged longevity. The constant production of new ramets may be a more successful mechanism for population regeneration given the unpredictability of rainfall events that lead to successful seedling establishment in the Plains of Magdalena. Nevertheless, clonal propagation may have a cost, especially if clones spread over large areas, increasing the probability of geitonogamy and biparental inbreeding

(Handel, 1985; Trame et al., 1995; Charpentier et al., 2000). Preliminary evidence indicate that the fecundity of genets is reduced when pollinators move pollen at distances below 20 m (Clark-Tapia, unpubl. data), and thus large genets may experience a reproductive cost.

Elasticity values of *S. eruca* at the genet and ramet level showed that ? values were proportionally more sensitive to stasis and growth than the observed values for other cacti (Godínez-Alvarez et al., 1999, Esparza-Olguín et al., 2002; Rae and Ebert, 2001). Silvertown et al., (1993) found that ? values in long-lived plants were characteristically most sensitive to changes in stasis, followed by the growth of adult individuals. The high rate of survival of genets (100%) and ramets (99%) as well as continuous growth to later stages, is contributing to the importance of these two processes to population growth (as shown by elasticity analysis) in *S. eruca*. Demographic processes, however, showed variable contributions to ? between years probably due to the highly variable environmental variables (i.e. rainfall event). According to Miriti et al., (2001) the temporal variation in abiotic variables may be an important mechanism for population persistence in desert environments.

Stenocereus eruca is considered as threatened under the Mexican legislation (SEDESOL, 2001), and demographic studies can provide guidelines for conservation programs of this species. We did not record evidence of disturbance by illegal collection or habitat destruction due to agricultural development (the major threats according to Cancino et al., 1995) in our permanent plots, nor in the area around each studied population. We did however observe evidence of plant burning and waste disposal in populations near human settlements (i.e. Poza Grande) and habitat destruction for agricultural development in the Villa Insurgentes-Ciudad Constitución agricultural area. Although these activities were not directly recorded in our demographic plots, we have observed evidence of disturbance that is very likely reducing and fragmenting the distribution of this narrow endemic species. *S. eruca* has a patchy distribution along a narrow coastal fringe (20 x 250 km) and human disturbance is likely to be the major threat to these populations.

Molecular evidence (Clark-Tapia 2000; R. Clark-Tapia, unpublished data) suggests that populations have moderate levels of genetic diversity and high levels of clonal diversity without any geographic pattern that could suggest the protection of particular areas of high diversity. Given that human disturbance is the major threat, and is likely to increase fragmentation in the near future, making *S. eruca* more vulnerable to extinction, we suggest the establishment of at least one reserve with protection from human disturbance. Estero Salinas could be a viable option for protection for two reasons: (1) It is the only large population (˜ 4700 ha) that it is relatively isolated and where we have not observed human disturbance and (2) it is the only population where we have regularly observed sexual reproduction (fruit and seed production), and would therefore have the potential of generating new recombination, and

maintaining genetic diversity. This population can be easily protected on a permanent basis and used as a source of plant material for restoration programs once disturbance has been reduced. Finally, we suggest the implementation of a program of environmental education in the region in order to reduce human disturbance and promote understanding of the Mexican legislation aimed at protecting threatened plants.

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Capitulo III
BIOLOGIA REPRODUCTIVA

Reproductive ecology of the rare clonal cactus, *Stenocereus eruca*, in the Sonoran desert.

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Abstract

Stenocereus eruca is a clonal cactus with an extremely narrow distribution in Baja California, in which seedling recruitment has rarely been observed. Low seedling recruitment in clonal plants may be caused by low seed production as a consequence of pollinator limitation or if seed input is sufficient, by lack of favorable conditions or microsites for seedling establishment. In this paper, we study the reproductive ecology of *S. eruca* along four years in order to explore the proximate causes of the low seedling recruitment observed in its populations. Flowers are self-incompatible, secrete up to 200 μL of nectar with sugar concentration of 21-23% and are predominantly nocturnal, with little opportunities for diurnal visitors. Major flower visitors were sphingids (*Hyles lineata* and *Erinnys ello*) and an unidentified native bee. The proportion of flowers setting fruit (fruit set) was in general low, with values ranging from 0.03 to 0.15 among four populations from 1999 to 2002. Bees were observed visiting flowers during 1999, 2001 and 2002 while sphingids were observed visiting the flowers only during 2000. Pollination treatments showed evidence of pollinator limitation during 1999, 2001 and 2002, but not during 2000, when sphingids were observed. Overall, sphingids seem to be unreliable and likely to be the missing pollinator responsible for the low fruit set observed among populations of *S. eruca*.

Key words: Clonal propagation, columnar cactus, pollination limitation, reproductive ecology, Sonoran desert, sphingids, *Stenocereus eruca*.

Sexual reproduction and vegetative propagation are two mechanisms through which plants regenerate their populations and achieve demographic stability (Harper 1977). Sexual reproduction produces new genetic recombination that allow adaptation to changing environments as well as the colonization of new habitats, while clonal propagation perpetuates successful genotypes in local populations (Abrahamson 1980). Clonal plants are common in a wide diversity of environments (Abrahamson 1980). In arid environments, sexual recruitments are often rare events in space and time while vegetative propagation often have a higher probability of establishment of ramets (Mandujano et al. 1998, Arizaga & Ezcurra 2002). It is not clear, however, what ecological scenarios have favored the evolution of clonal propagation in particular lineages of desert plants. Models employing game theory predict that plants should allocate a larger resource fraction to clonal propagation when the cost of seed production relative to ramet production is high and the establishment probability of ramets relative to seed is also high (Olejniczak 2001).

Columnar cacti exhibit considerable variation in their mechanism of regeneration, from those relying only on sexual reproduction to those showing a combination of sexual and clonal mechanisms (Bravo-Hollis 1978, Gibson 1989, Parker 1989). Breeding systems are usually self-incompatible in columnar cacti and pollen transfer is

mediated by a wide diversity of animal vectors (Boyle 1997, Valiente-Banuet et al. 1996). Among Mexican columnar cacti species, floral morphology suggest that most species (c.a. 60%) are bat-pollinated while the rest have pollination syndromes that include the participation of hummingbirds, bees and hawkmoths (Valiente-Banuet et al. 1996). Pollination studies on chiropterophilous species conducted within and outside the tropics have revealed clear geographic patterns. Columnar cacti from tropical deserts show specialized pollination systems with bats as the major pollinators, whereas species outside the tropics exhibit moderate generalized systems, being pollinated by both bats and diurnal visitors, presumably due to the unpredictability of bat activity (Valiente-Banuet et al. 1997, Fleming et al. 2001). However, it is not clear whether pollinator unpredictability has caused similar trends in the pollination systems of species with other pollination syndromes, or if clonal propagation could evolve under those conditions. Studies on the pollination biology of *Lophocereus schottii* have shown evidence of moth specialization in the Sonoran desert (Fleming & Holland 1998). Thus, the geographic trends in the pollination systems that have been described in chiropterophilous species may not hold for columnar cacti with other pollination syndromes. Nevertheless, our knowledge about the reproductive biology of columnar cacti pollinated by hummingbirds, bees and hawkmoths is quite limited. In addition, nothing is known about the relative role of sexual recruitment *vs*. clonal propagation in the population regeneration of clonal columnar cacti.

Stenocereus eruca is a clonal columnar cactus of the Sonoran desert (Gibson 1989, Turner et al. 1995, Fig 1). It is an extremely narrow endemic species, restricted to the coastal areas of the Plains of Magdalena in Baja California (Gibson 1989, Fig 1). This species has been cited as the most remarkable case of clonal propagation in the Cactaceae (Gibson & Nobel 1986). It has a procumbent growth habit with horizontal stems growing along the ground (Fig 2). Clonal propagation occurs by detachment of branches from the major shoot as the base of the branch dies and rots. Field observations have been unable to detect seedlings of sexual origin and it is thought that population regeneration occurs mainly through clonal propagation (Gibson 1989, Turner et al. 1995). The flowers of *S. eruca* are nocturnal and its morphological features suggest pollination by hawkmoths (Gibson 1989, Turner et al. 1995). However, our knowledge about the pollination biology and the role of sexual reproduction in the regeneration in *S. eruca* is limited.

Low seedling recruitment in clonal plants may have distinct underlying mechanisms (Mandujano et al. 1996, Eriksson 1997). Seedlings may be extremely rare due to low seed production as a consequence of low pollinator availability (Bierzychudek 1981), or if seed production is not limited, by lack of favorable conditions or microsites for seed germination and establishment (Mandujano et al. 1996, 1998). Thus, sexual recruitment in *S. eruca* may be limited by low seed production due to low pollinator availability, a high spatio-temporal variation in pollinator abundance, or by the lack of favorable microsites for seedling establishment. We studied the reproductive ecology of *S. eruca* in order to explore the proximate causes of the low seedling recruitment observed in its populations. First, we explore the spatial and temporal variation in fruit set (fruit/flower ratio) among populations in order to evaluate seed production success. Second, we describe basic aspects of the pollination biology of this species and analyze the temporal variation in pollinator visitation. Third, we explore whether female fecundity is pollinator limited through a series of pollinations experiments.

Material and Methods.

Temporal and spatial variation in fruit set.- We selected four populations trying to cover the entire distribution range of *S. eruca* (Fig. 1). In each population we established a permanent plot (10 x 60 m) within which we tagged and marked all ramets (270-300). *Stenocereus eruca* flowers from August to October (Gibson 1989, Turner et al. 1995). During 1999, 2000, 2001 and 2002 we visited each

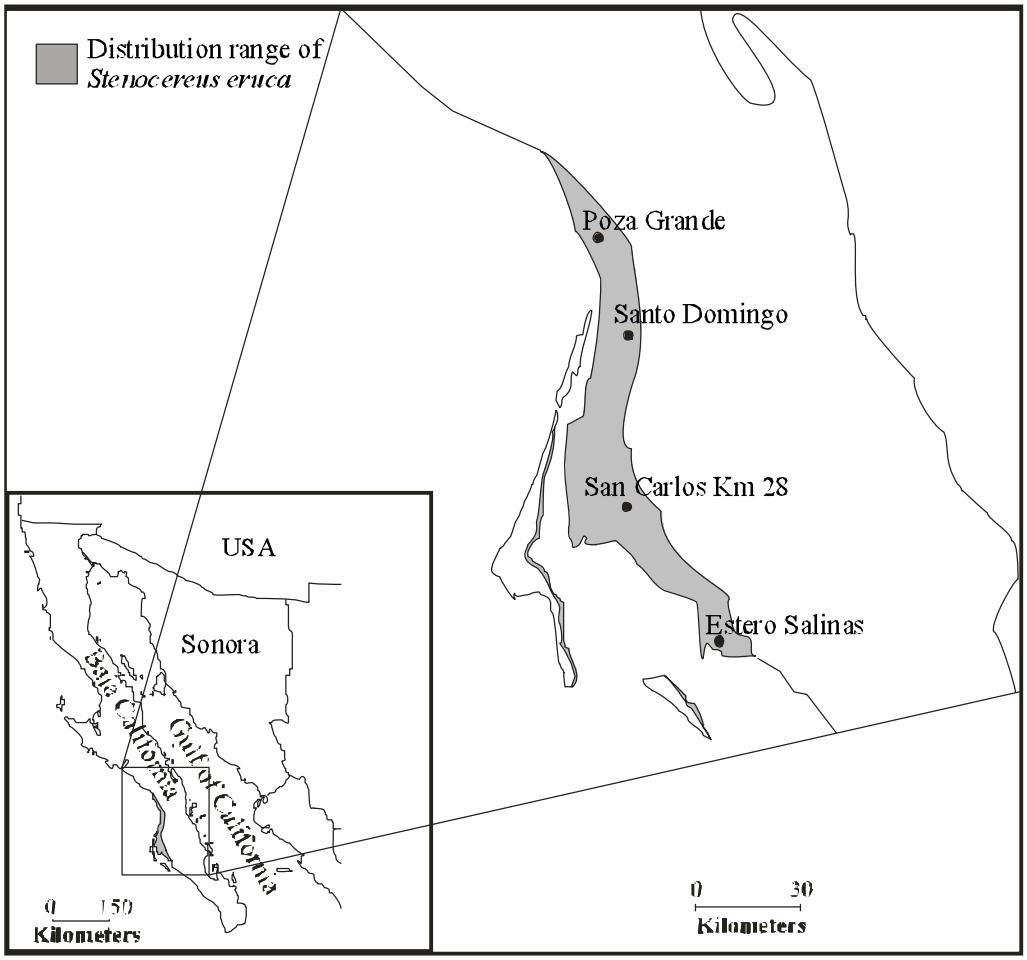

Fig. 1. Range of distribution (shaded area) of *Stenocereus eruca* in Baja California and the location of the studied populations. Modified from Turner et al., (1995).

Fig. 2. Growth habit of *Stenocereus eruca* growing at Estero Salinas, in Baja California.

population at the end of the reproductive season (November-December) to record flower and fruit production per ramet and per plot (area basis). Abortive flowers and mature fruits usually leave dry tissue fragments on the areoles where they were produced and they may be found for long periods of time, allowing to count the number of flowers and fruits produced per ramet and per plot.

Pollination biology.- We studied the pollination biology of *S. eruca* only at Estero Salinas because this population was the only one that produced sufficient numbers of

flowers (see Table 1). During 9-10 August of 1999, 21-22 September of 2000, 9-10 August of 2001 and 19-20 September of 2002 we marked nine flowers from nine ramets to describe flower anthesis. Flower anthesis was described by measuring with a caliper the distance between opposite perianth tips every hour, beginning at bud opening and up to flower closure. We recorded the number and type of flower visitors during two nights every year. During 1999 we observed six focal flowers at 5-min intervals every hour and 10 focal flowers per night during 2000, 2001 and 2002. Flower visitors were collected with hand-nets and prepared for later identification. We observed whether visitors made contact with anthers and stigmas. During 1999 and 2000 we measured potential pollen movement by using fluorescent dyes applied to anthers. We applied fluorescent dye to anthers of two focal flowers and scored the presence of dye in samples of 4 flowers located at 5, 10, 20, 50, 100 and 300 m using UV light.

We measured nectar volume and sugar concentration only during the year 2000. Flower buds were bagged with bridal veil netting during the afternoon to avoid nectar removal by visitors. Flowers of *S. eruca* are one of the longest in the genus *Stenocereus*, reaching up to 15 cm (Gibson 1989) and are often curved. Preliminary measurements indicated that nectar extraction with microcapillar tubes was extremely difficult. Thus, we had to dissect flowers in order to measure nectar accumulation. We used a total of 56 flowers to measure nectar accumulation during the night. Every two hours, beginning at bud opening, we dissected eight flowers and extracted and measured the accumulated nectar using 100 µL capillar tubes. Sugar concentration was also measured every two hours using a low volume field refractometer (0-50%, Bellingham & Stanley).

Pollination experiments.- In order to determine whether flowers are self-compatible and whether female fecundity is pollinator-limited, we applied three pollination treatments during 1999, 2000, 2001 and 2002 at Estero Salinas. Flower production per ramet was rather low (see Table 1); thus we were able to use only one flower per ramet for the pollination treatments. We employed a total of 132, 124, 140 and 119 flowers for the three pollination treatments during 1999, 2000, 2001 and 2002, respectively. The pollination treatments were as follows: a) Manual-cross pollination (MCP): flower buds were bagged with bridal veil netting; when flowers opened they were hand pollinated by rubbing the stigma with a set of anthers containing fresh pollen obtained from another ramet at least 100 m away. b) Open-pollination (OP): flowers that opened during three consecutive nights and were available to any visitor were tagged and followed. c) Manual self-pollination (MSP): flower buds were bagged during the afternoon; when flowers opened and pollen was released, they were hand-pollinated by rubbing the stigma with anthers with fresh pollen from the same flower and bagged. The fate (*i.e.* aborted or developing fruit) of the tagged flowers from the pollination treatments was recorded 45 days later. Mature fruits were collected and once in the lab they were opened and the number of seeds per fruit was counted. Fruit set values between pollination treatments were compared through chi-square tests while the number of seeds per fruit was compared using t-tests with the JMP 3.1 software (SAS Institute 1997). Seed germination was evaluated only for seeds from open-pollinated flowers in 1999, 2000 and 2001. Seeds were placed in petri dishes containing 2% agar at 25 °C in a growth chamber for 30 days using five replicates of 50 seeds.

Results

Temporal and spatial variation in fruit set.- Ramets produced on average from 0.06 to 2.5 flowers and from 0.003 to 0.130 fruits. The number of reproductive structures observed per plot (600 m^2) ranged from 18 to 661 flowers and from 1 to 35 fruits in different populations and years (Table 1). Southern populations produced on average more

Table 1. Flower and fruit production per ramet and plot in four populations of *Stenocereus eruca* from the Plains of Magdalena, Baja California Sur, during 1999, 2000, 2001 and 2002. Values shown are means ± 1 SD. Plots had 600 m^2 (10 x 60 m).

Population	Flower production during				Fruit production during			
	1999	2000	2001	2002	1999	2000	2001	2002
Estero Salinas								
Mean/ramet SD	1.886 (±3.450)	2.457 (±3.306)	1.891 (±2.882)	1.410 (±1.561)	0.057 (±0.264)	0.130 (±0.490)	0.065 (±0.275)	0.058 (±0.262)
Total production/plot	496	661	522	369	15	35	18	16
Fruit/flower ratio					0.030	0.053	0.034	0.043
San Carlos								
Mean/ramet SD	0.327 (±1.063)	0.498 (±1.074)	0.664 (±1.969)	0.770 (±1.663)	0.029 (±0.207)	0.048 (±0.258)	0.041 (±0.230)	0.026 (±0.178)
Total production/plot	90	145	196	238	8	14	12	8
Fruit/flower ratio					0.089	0.097	0.061	0.034
Santo Domingo								
Mean/ramet SD	0.212 (±0.992)	0.331 (±0.849)	0.174 (±0.812)	0.186 (±0.816)	0.007 (±0.085)	0.018 (±0.133)	0.011 (±0.103)	0.013 (±0.115)
Total production/plot	58	92	49	56	2	5	3	3
Fruit/flower ratio					0.034	0.054	0.061	0.054
Poza Grande								
Mean/ramet SD	0.062 (±0.444)	0.160 (±0.744)	0.138 (±0.618)	0.142 (±0.552)	0.003 (±0.059)	0.024 (±0.209)	0.007 (±0.082)	0.006 (±0.080)
Total production/plot	18	47	41	44	1	7	2	2
Fruit/flower ratio					0.056	0.149	0.049	0.045

flowers and fruits (Table 1). Fruit set per plot ranged from 0.03 to 0.15 among populations and years, with no clear geographic pattern (Table 1).

Pollination biology.- Flower anthesis was essentially nocturnal with minor fluctuations among years and narrow opportunities for diurnal visitors (Fig 3a). Nectar accumulation was gradual during the night reaching up to 150 µL by 2:00 and close to 200 µL by 7:00 (Fig 3b) while sugar concentration remained almost constant, varying from 21% to 23% (Fig 3c). The type of flower visitors varied between years (Fig 3d). During 1999, 2001 and 2002, we recorded an unidentified native bee species visiting the flowers before sunset and after sunrise; visitation rates ranged from 0.8 ± 0.8 to 8.5 ± 3.9 visits/flower/5 min (Fig 3d). Bees collected pollen before sunset and usually contacted stigmas. During 1999 when only bees were observed, the proportion of flowers with fluorescent dye at 5 and 10 m from focal flowers was 1.00 and 0.5, and 0.0 for

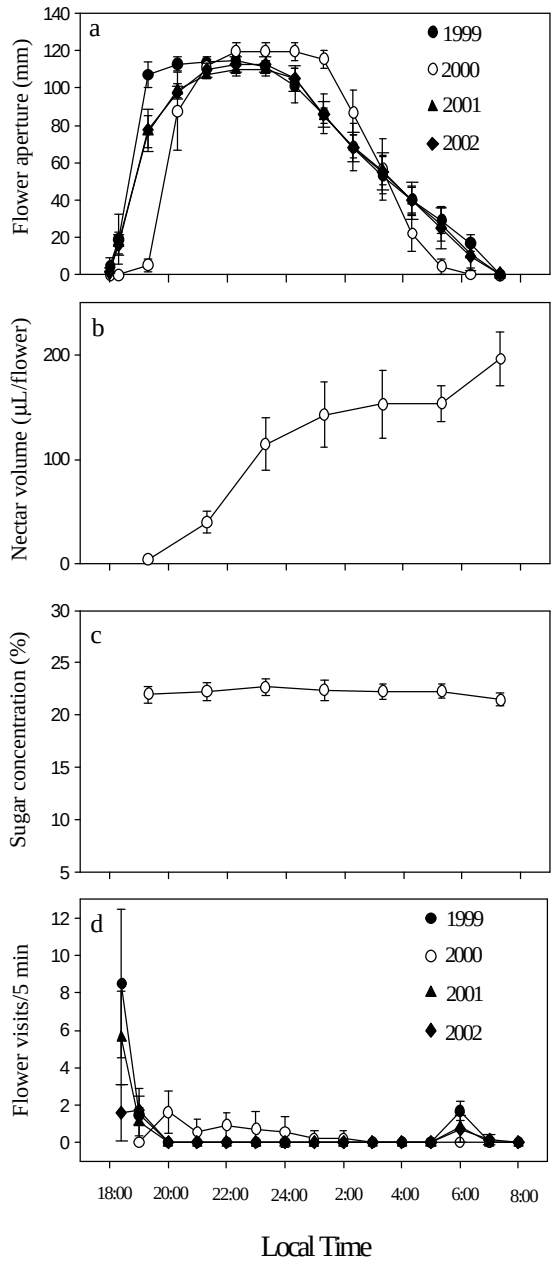

Fig.3. Temporal pattern of flower opening and closing, nectar accumulation, sugar concentration and visitation rates to flowers of *Stenocereus eruca* at Estero Salinas. a) Dynamics of flower aperture and closure during 1999, 2000 and 2001. b) Nectar accumulation in flowers during the year 2000. c) Sugar concentration (Brix) in nectar accumulated in flowers during the year 2000. d) Visitation rates to flowers of *Stenocereus eruca* during 1999, 2000 and 2001.

flowers located at greater distances. During 2000, we recorded two species of sphingids (*Hyles lineata* and *Erinnys ello*) visiting the flowers between 19:00 and 1:00, with visitation rates ranging from 0.2 ± 0.4 to 1.6 ± 1.1 visits/flower/5 min (Fig 3d). Sphingids always contacted anthers and stigmas during the night. During 2000, when only sphingids were observed, the proportion of flowers with fluorescent dye was equal or greater than 0.5 for flowers located up to 100 m from focal flowers and 0.25 for flowers located at 300 m.

Pollination treatments.- None of the manually self-pollinated flowers set fruit, indicating self-incompatibility (Fig 4). Significant differences were detected between fruit set in the manual cross-pollination (MCP) and open-pollination (OP) treatments during 1999 (χ^2=17.4, P<0.0001), 2001 (χ^2=15.2, P<0.0001) and 2002 (χ^2=15.4, P<0.0001), while for 2000, no significant differences were detected between these two treatments (χ^2=2.5, P=0.11), indicating pollinator limitation for 1999, 2001 and 2002 but not for 2000. In contrast, no significant differences were detected in relation to the number of seeds/fruit between the MCP and OP treatments during 1999 (t=0.78, P=0.44) and 2000 (t=0.54, P=0.59) while clear differences were detected for 2001(t=2.18, P=0.03) and 2002 when the OP treatment did not set any fruit (Fig 4). Seed germination from open-pollinated flowers was 36% (± 18%), 55% (± 29%) and 28% (± 30%) during 1999, 2000 and 2001, respectively.

Discussion

In this paper we have shown that flowers of *S. eruca* are self-incompatible and are visited by sphingids and bees. Evidence from pollination treatments and spatial and temporal variation in fruit set seem to indicate that pollinators limit female fecundity. Although our field observations were quite limited, overall, our data suggest that sphingids are better pollinators but unreliable in time, which points towards this group as the likely missing

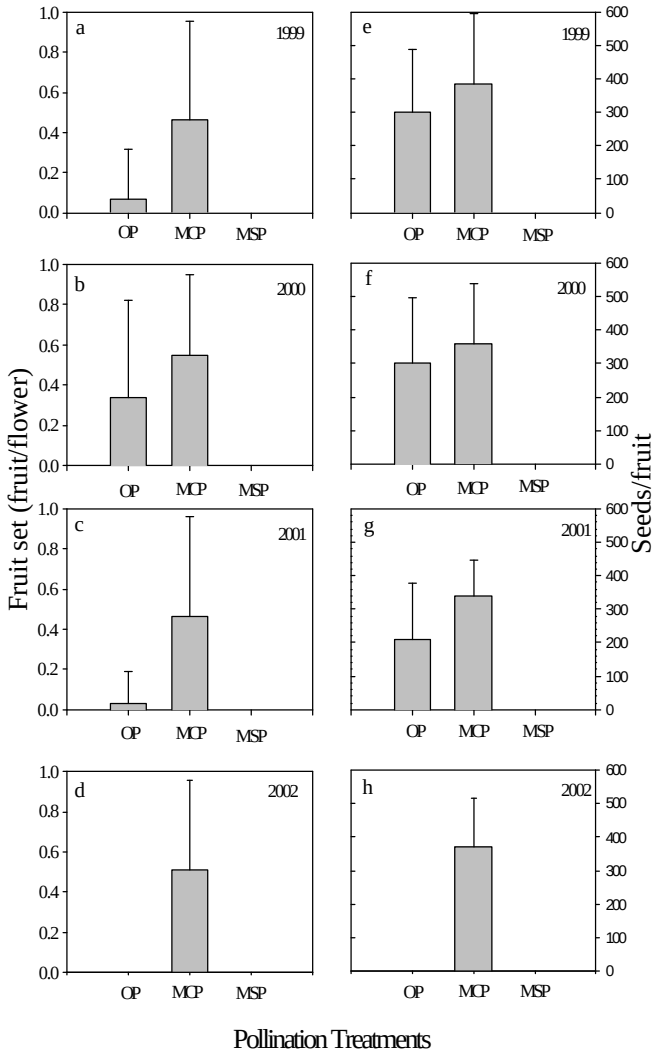

Fig. 4. Fruit set and the number of seeds per fruit for the different pollination treatments applied to flowers of *Stenocereus eruca* during 1999, 2000, and 2001. Values shown are means + 1 SD.

pollinator responsible for the low fruit set observed among populations.

Our results show some similarities and some striking differences when compared to other columnar cacti. As described for other members of the genus (Boyle 1997), *S. eruca* exhibit self-incompatibility. Also, the observed sugar concentration in its nectar falls within the range of other cacti pollinated by sphingids (Scogin 1985). As suggested by its flower morphology (Gibson 1989), it is visited by sphingid moths. However, fruit set values observed in different populations and years were in general lower than open-pollination values detected for other columnar cacti from the Sonoran desert (Fleming et al. 1996, 2001, Fleming and Holland 1998). The comparison with the moth-specialized *Lophocereus schottii* is revealing as in this case open-pollinated flowers usually set more than 40%, as a consequence of the reliable activity of the nocturnal moth, *Upiga virescens* (Fleming and Holland 1998). In contrast, *S. eruca* show low fruit set values probably as a consequence of the unpredictable activity of sphingids and the low efficiency of bees. Although we did not measure the efficiency of bees as pollinators, the low levels of fruit set observed in the open pollinated control treatment during the years where sphingids were not observed suggest low efficiency in the transfer of compatible pollen.

Female fecundity seems to be limited by pollinators in *S. eruca*. The evidence from the pollination experiment revealed significant differences in fruit set between the MCP and OP treatments during 1999, 2001 and 2002, when only bees were detected visiting the flowers. In contrast, during 2000, when sphingids were observed visiting the flowers, no significant differences were detected between treatments. Nevertheless, formal tests of pollinator limitation usually require the utilization of all the flowers produced per individual plants (Parra-Tabla et al. 1998). Although we did not use all the flowers produced per individual genets, we employed most flowers produced by individual ramets. As ramets are unconnected, there is no re-translocation of resources. Thus, our evidence suggests limitation by pollinators, at least during the time period of our pollination treatments in 1999, 2001 and 2002. On the other hand, the similarity of fruit set values observed for most populations and years with the values of the control treatments of 1999, 2001 and 2002, suggest that pollinator limitation was responsible for the low fecundity detected in *S. eruca* in these years. Furthermore, the clear differences in fruit set between the MCP treatment (all years) and the OP

treatment of 2000 with respect to the variation in natural fruit set values among populations and years (Table 1), suggest that sphingid activity was quite restricted in space and time. Sphingid activity is known to be sensitive to temperature conditions (Martínez del Río and Búrquez 1986; Casey 1993). During the brief periods when we observed sphingids at Estero Salinas, we noticed that they are active only when night temperature is above 18 °C. A preliminary analysis of the minimum temperatures of August and September at a station near Estero Salinas revealed great variation between years (Clark-Tapia, unpubl. data). Thus, the low and unreliable activity of sphingids during the flowering time of *S. eruca* might be associated with variation in night temperatures. However, our evidence is limited and a formal test will require a detailed field study of sphingid activity and night temperatures for a longer period of time.

The clonal structure of plant populations is known to have important consequences on their reproductive success and mating system (Handel 1985, Charpentier et al. 2000). The fact that *S. eruca* is self-incompatible may imply that all pollen transfer between ramets of the same genet will cause pollen waste and probably a reduction of seed set due to stigma saturation (de Jong et al. 1992). Our preliminary data on pollen transfer using fluorescent dyes in this species suggest that bees frequently move pollen at distances < 10 m, while sphingids often move pollen at distances > 10 m. Thus, if genets of *S. eruca* are spread across large areas, the probability of pollen transfer within the same genet would be greater for bees than for sphingids, which are known to carry out relatively longer-distance flights (Janzen 1984). A formal study of pollen supplementation (Charpentier et al. 2000) would allow further exploration of the effect of clonal structure on the mating system of *S. eruca*.

In conclusion, our results show that seed production is low most likely due to pollinator limitation. Nonetheless, although seed input is low, seeds are available for germination every year. Thus, low seedling recruitment in *S. eruca* is probably due to lack of favorable conditions or microsites for seed germination and establishment. In order to make further progress, future studies should examine factors affecting seedling establishment and the role of sexual *vs.* clonal recruitment in the demography and clonal structure of *S. eruca*.

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Efectos de la estructura clonal sobre el sistema reproductivo de la cactácea clonal *Stenocereus eruca*

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Resumen

Stenocereus eruca es una cactácea columnar postrada endémica del Desierto Sonorense que se regenera principalmente por propagación clonal. En este capítulo se examina la manera en ~~la~~ que el arreglo espacial de ramets, la incompatibilidad y la variación espacio-temporal de polinizadores afectan al sistema reproductivo de esta especie-a través del efecto potencial en los niveles de geitonogamia (polinización entre flores del mismo genet). Se montó un experimento de suplementación de polen a través de cinco tratamientos con polen colectado a 1, 10, 100, 1000 y 25000 metros de distancia de las flores receptoras durante los años 2001 y 2002, con la finalidad de conocer los efectos de la estructura clonal sobre el sistema reproductivo. Los resultados de este estudio revelan que *Stenocereus eruca* presenta una reducción significativa de la fecundidad femenina cuando la polinización ocurre entre individuos situados a cortas distancias (1 y 10 metros de distancia), al parecer como producto de geitonogamia y endogamia biparental. Se sugiere que el arreglo espacial agregado de los ramets de un mismo genet y la variación espacio-temporal en la abundancia y tipo de polinizadores pueden causar altos niveles de geitonogamia, endogamia biparental y ser la causa proximal del bajo reclutamiento sexual-observado en las poblaciones de *S. eruca*.

Palabras claves: Estructura clonal, Geitonogamia, Polinizadores, Propagación clonal, sistema reproductivo, *Stenocereus eruca*.

Introducción

La gran mayoría de las especies clonales combinan la reproducción sexual con la propagación clonal como mecanismo de regeneración de sus poblaciones (Abrahamson, 1980; Richards, 1986). La combinación de ambas formas de propagación, puede tener consecuencias evolutivas y ecológicas importantes para las especies involucradas (Silander, 1985; Eriksson, 1997; Charpentier, 2002; Eckert, 2002). No obstante, el propagarse clonalmente le confiere a las especies numerosas ventajas ecológicas, tales como la capacidad de colonizar rápidamente un área debido al crecimiento en tamaño de un clon (*i.e.* expansión y consecuente fragmentación) (Cook, 1985; Cain,1990), la facultad de explorar el ambiente dentro de su hábitat (Pitelka y Ashmun, 1985; Cain *et al*. 1996; Oborny y Cain, 1997) y el potencial de permanecer por un largo periodo de tiempo, debido a la capacidad ilimitada de crecimiento (Orive, 1995; de Kroon y van Groenendael, 1997). Diversos estudios sobre biología de la polinización en plantas clonales han documentado que la estructura clonal a nivel espacial y la integración fisiológica entre ramets pueden tener implicaciones evolutivas y ecológicas importantes sobre la función y mantenimiento de la reproducción sexual, como consecuencia de su posible influencia sobre la frecuencia de eventos de geitonogamia, es decir, eventos de polinización entre flores de la misma rama, entre flores de una planta o entre flores de diferentes ramets de un genet (Handel,

1985; Trame *et al.* 1995; Charpentier *et al.* 2000; Eckert 2000; Charpentier, 2002).

Se ha sugerido que la expansión de los genets de una especie clonal puede incrementar los riesgos de depresión endogámica como consecuencia de altos niveles de geitonogamia (de Jong, *et al*. 1993, Trame *et al*, 1995), ocasionando además un alto costo en términos de desperdicio de polen y mostrando efectos negativos en la producción de semillas, debido a una saturación del estigma, obstrucción del estilo y abortos de los óvulos autopolinizados (de Jong *et al*. 1992). Las poblaciones de especies de plantas clonales se caracterizan por presentar eventos de reclutamiento sexual esporádicos o restringidos, lo cual frecuentemente se atribuye a la variabilidad ambiental o a limitación de polinizadores (Eriksson, 1993; Jones y Gliddon, 1999; Verburg *et al.*, 2000). La ausencia o limitación de reclutamientos sexuales en especies clonales autoincompatibles podría también ser producto de altas tasas de geitonogamia (Handel, 1985; Trame *et al.* 1995; Charpentier *et al.* 2000; Charpentier, 2002), distancias de entrecruzamiento no optimas (correlación inversa entre similitud genética y distancia geográfica, Waser y Price, 1989; Waser y Price, 1991; Trame *et al*. 1995) o de depresión por exogamia (producto de cruzas entre individuos adaptados a diferentes condiciones ambientales, Waser, 1993; Waser y Price, 1994). Existen muy pocos estudios que aborden los efectos de altos niveles de geitonogamia, estimaciones de distancias de entrecruzamiento optimas y depresión por exogamia en la fecundidad de especies clonales.

Stenocereus eruca es una cactácea clonal del Desierto Sonorense (Gibson, 1989, Turner *et al.* 1995). Esta especie presenta un área de distribución muy restringida, siendo endémica de las planicies de Magdalena en Baja California Sur (Gibson, 1989, Fig 1). Las flores de *S. eruca* son auto-incompatibles y la producción de frutos es baja y altamente variable en espacio y tiempo, posiblemente a consecuencia de una limitación de polinizadores asociada a una variación en la abundancia de esfíngidos y a la presencia de polinizadores poco efectivos, tales como abejas (Capítulo 3). En más de una década de observación, nunca se ha observado el reclutamiento de plántulas en poblaciones naturales de *S. eruca*, en tanto que se ha visto que la propagación clonal es la principal estrategia de regeneración de sus poblaciones (Gibson, 1989, Turner *et al.* 1995, Clark-Tapia, 2000).

Gibson y Nobel (1986) han sugerido que *S. eruca* es uno de los casos más extremos de propagación clonal dentro de la familia Cactáceae. Sin embargo, la evidencia disponible sobre la diversidad genotípica muestra valores que permiten inferir que la reproducción sexual es una fuente importante de regeneración poblacional (Clark, 2000; Capítulo 1). En esta especie, la propagación clonal se da por medio de la fragmentación de ramas del eje principal, mientras que la parte basal muere y se desintegra. Estudios previos utilizando isoenzimas (Clark-Tapia, 2000) y RAPDs (Capítulo 1) sugieren que las poblaciones presentan una estructura clonal en la que los ramets de un mismo genet pueden estar densamente agregados o bien ampliamente distribuidos dentro de la población. Este tipo de distribución espacial de individuos con un mismo genotipo podría tener un efecto significativo en la fecundidad femenina, dado el sistema de incompatibilidad de esta especie.

La estructura espacial de los ramets y genets, así como la variación espacio-temporal en la abundancia de polinizadores pueden tener efectos importantes en el sistema reproductivo de *S. eruca*; concretamente en las tasas de reclutamiento sexual dentro de las poblaciones de esta especie. Para probar esta hipótesis, se realizaron experimentos de suplementación de polen entre individuos localizados a diferentes distancias y se llevaron a cabo análisis de identidad genética utilizando

RAPDs como marcador molecular. El análisis genético del donador y receptor en el experimento de suplementación de polen permitió evaluar por un lado el efecto de la similitud genética sobre el éxito de las cruzas, como función de la distancia, y por otro el éxito de las cruzas como función de las bandas compartidas en los RAPD´s entre pares de individuos.

Material y Métodos

Se llevó a cabo un experimento de suplementación de polen en la población de Estero Salinas en las Planicies de Magdalena, Baja California Sur, México (Fig. 1). Se seleccionó esta población debido a que presenta una densidad de ca. 3000 ramets por hectárea y ha sido la única que ha presentado un número considerable de flores durante las tres temporadas en las que se ha estudiado la biología reproductiva (ver Capítulo 3a).

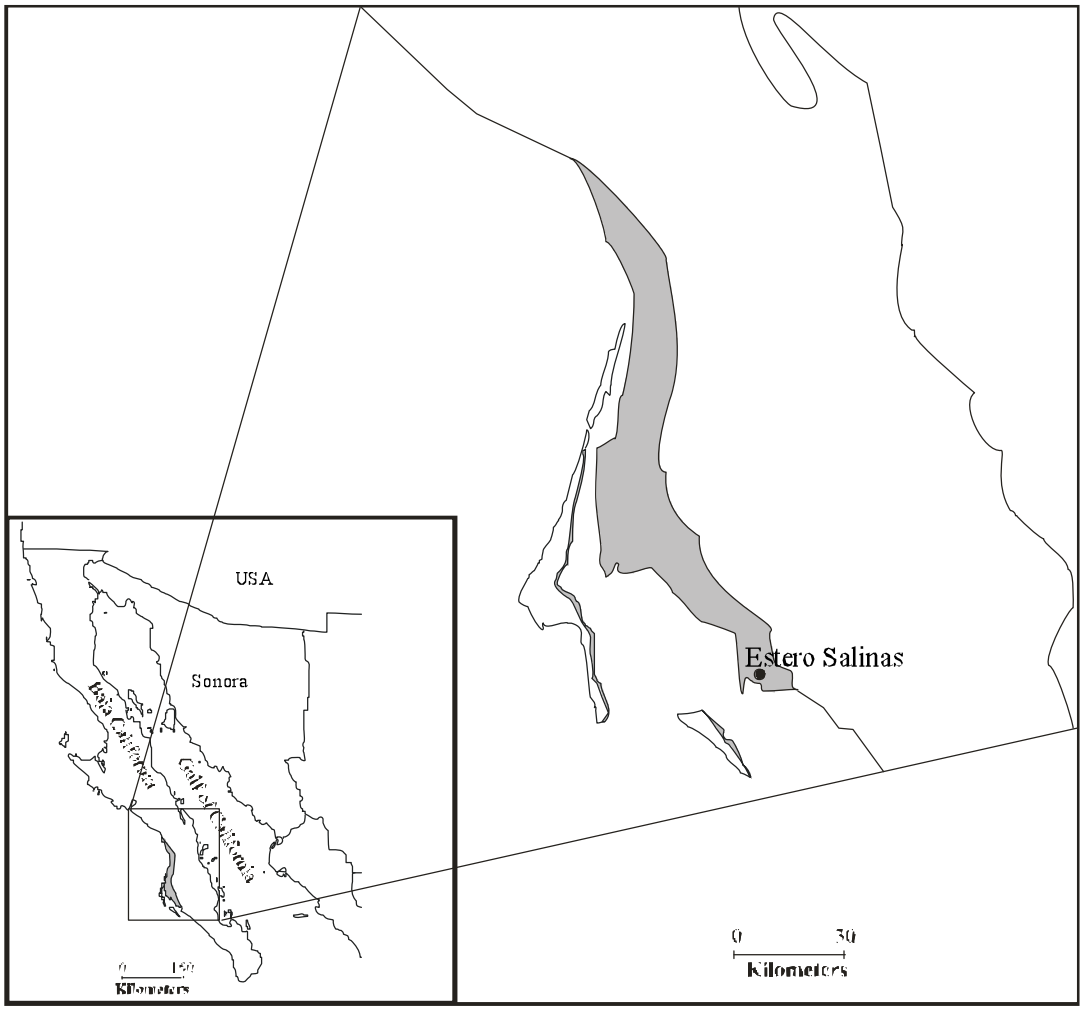

Fig. 1.- Distribución geográfica (área sombreada) de *Stenocereus eruca* y localización de la población estudiada. Modificado de Turner *et al*. 1995.

Experimentos de suplementación de polen

Estos experimentos se realizaron durante los meses de agosto a noviembre de los años 2001 y 2002, ya que la floración y fructificación de la especie en estudio normalmente ocurre en esos meses. Para examinar los efectos de la geitonogamia y la distancia de entrecruzamiento en el sistema reproductivo de *S. eruca*, se seleccionaron al azar 60 y 80 individuos reproductivos en la población de estudio (2001 y 2002, respectivamente). En agosto de 2001 y 2002, cada uno de estos individuos se polinizó manualmente con polen de donadores provenientes
de individuos localizados a cinco distancias (1, 10, 100, 1000 y 25,000 m.). El polen localizado a 25,000 m de distancia se colectó y transportó de una segunda población localizada en San Carlos, con la finalidad de evaluar si se presenta algún tipo de depresión por exogamia (Fig. 1).

La suplementación de polen de cada categoría de distancia se aplicó a las flores de los individuos seleccionados, hasta completar un mínimo de 10 y 20 replicas (flores) por tratamiento, durante los años 2001 y 2002, respectivamente. Las flores de cada tratamiento se cubrieron con bolsas de malla durante todo el experimento con la finalidad de evitar las visitas de los polinizadores. En noviembre (época de la fructificación) se estimó la adecuación de los individuos de cada uno de los tratamientos por medio de la evaluación de: a) la producción de frutos (proporción de flores que producen frutos), b) la producción promedio de semillas por fruto, c) la fecundidad total (producción de frutos multiplicado por el número de semillas), y d) el porcentaje de germinación de las semillas obtenidas de cada tratamiento. Para evaluar el porcentaje de germinación, se colocaron en cajas de petri con agar (2%) 100 semillas provenientes de cada tratamiento de polinización en una cámara de crecimiento a 25°C durante tres meses. Cada tratamiento consistió de 7 replicas (para dar un total de 700 semillas por tratamiento). Asimismo, se estimó el porcentaje de germinación de un tratamiento control (semillas obtenidas en 2001 a partir de polinización natural) para evaluar si existen diferencias en la capacidad germinativa de estas con respecto a las obtenidas en los tratamientos de suplementación.

Análisis de identidad genética

Para examinar la identidad genética de cada donador y receptor de polen, se colectó una porción de clorenquima de los individuos reproductivos seleccionados para los experimentos de suplementación de polen, así como de los individuos donadores de polen. Los detalles de colecta, conservación y análisis genético de muestras con Random Amplified Polymorphic DNA (RAPDs) son similares a los utilizados en el Capítulo 1 de este trabajo. La similitud genética entre cada par de individuos (donador-receptor de polen) dentro de cada unidad experimental, se determinó contando el número de bandas compartidas en el análisis de RAPDs con seis primers. En base al número de bandas compartidas entre pares de individuos (donador-receptor) se calculó el índice de similitud genética de Nei y Li´s (1979) calculado como $SG = 2m_{xy}/(m_x+m_y)$, donde m_{xy} es el numero total de bandas compartidas entre pares de individuos, mientras m_x y m_y son el número total de bandas presentes en una muestra. Esta medida de similitud genética permitió determinar si cada par de individuos (donador-receptor) analizados pertenecían al mismo genet, eran individuos completamente diferentes o parientes cercanos. Para determinar lo anterior, se consideró que los pares de individuos con valores de $S_G = 1$ eran el mismo genet, valores de $S_G < 0.75$ eran individuos diferentes y valores de $S_G > 0.75$ eran individuos genéticamente emparamentados.

Para determinar si existían diferencias significativas en la adecuación de los individuos de cada tratamiento (en términos de la producción de frutos, número promedio de semillas por fruto, fecundidad total y

porcentaje de germinación de semillas) por tratamiento, se utilizó una ANOVA. Se realizó un análisis de regresión lineal para evaluar si había una asociación entre la distancia geográfica y la distancia genética (similitud genética), así como entre la distancia genética y la eficiencia reproductiva (fecundidad total). Por último, se efectuó una regresión múltiple para determinar si existía una relación entre la eficiencia reproductiva (fecundidad total) de los individuos de cada tratamiento de suplementación de polen con la distancia focal entre cada par de individuos (donador-receptor) y su respectiva similitud genética. Los análisis estadísticos se realizaron con el programa SigmaStat v. 2.01.

Resultados

La producción de frutos (proporción de flores que producen frutos) varió entre las cinco distancias de suplementación de polen, de 0±0.00 (media ± 1 EE) a 0.80±0.13 y de 0.1±0.07 a 0.68±0.11 en el 2001 y 2002, respectivamente (Tabla 1). Los resultados del experimento de suplementación de polen mostraron que las cruzas entre individuos localizados a 1 y 10 m de distancia en 2001 no dieron lugar a frutos. Los resultados también muestran que la eficiencia reproductiva (producción de frutos) de los individuos que recibieron polen de una distancia de 1 y 10 metros fue significativamente menor que la de los individuos que recibieron polen de una distancia de 100, 1000 y 25,000 m, en los que la eficiencia reproductiva fue mayor al 50% en ambos años de estudio. Aún cuando no se encontraron diferencias significativas entre los resultados de estos últimos tres tratamientos en ninguno de los dos períodos de estudio, la producción de frutos fue mayor cuando el polen provino de una distancia de 1000 m de distancia y tendió a decrecer en los tratamientos de 25,000 m (Fig. 2A).

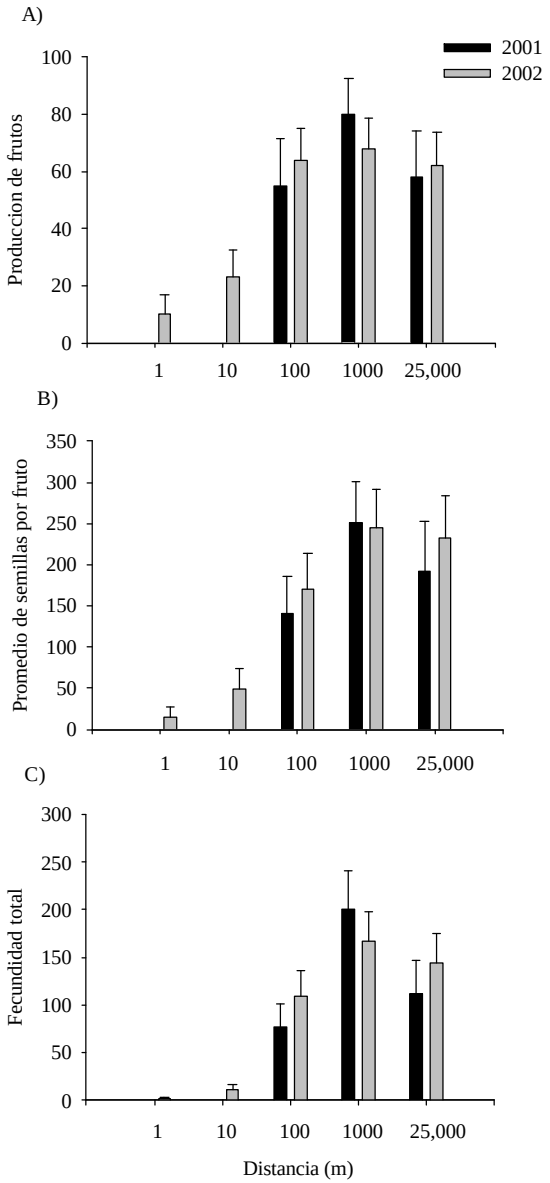

Fig. 2.- A) Producción de frutos (proporción de flores que producen frutos), B) producción promedio de semillas por fruto, y C) fecundidad total en los cinco tratamientos (1, 10, 100, 1000 y 25000 m) de suplementación de polen en Estero Salinas. Las barras representas el error estándar. Los tratamientos con la misma letra no difieren significativamente entre ellos cuando se comparo con una prueba de Tukey´s (ANOVA, P = 0.05). Los tamaños de muestra en el 2001 fueron de 10 individuos por tratamiento, mientras en el 2002, fueron de 20 individuos.

La producción promedio de semillas por fruto entre las distancias de los tratamientos de suplementación de polen varió de 0±0.00 a 251.2±49.32 y de 14.9±12.0 a

244.64±46.57 durante el 2001 y 2002, respectivamente (Tabla 1). Los resultados con respecto a la producción de semillas por fruto mostraron un patrón similar al observado en la eficiencia reproductiva. Los tratamientos de 1 y 10 m de distancia difirieron significativamente de los de 100, 1000 y 25000 m y las diferencias en número promedio de semillas entre estos tres últimos tratamientos no fueron significativas. Nuevamente los valores más altos de número de semillas por fruto se obtuvieron en el tratamiento de 1000 m de distancia, con un ligero decremento en el de 25,000 m (Fig. 2B)

La fecundidad total entre las distancias de tratamiento de suplementación de polen varió de 0±0.00 a 200.96±39.46 y de 1.49±1.20 a 166.36±31.66 durante el 2001 y 2002, respectivamente (Tabla 1). Los resultados con respecto a la fecundidad total mostraron un patrón similar al observado en las dos estimaciones de adecuaciones anteriores. Los tratamientos de 1 y 10 m de distancia difirieron significativamente de los de 100, 1000 y 25000 m. Sin embargo, las diferencias entre estos tres últimos tratamientos, en contraste con la producción de frutos y de la producción promedio de semillas por

Tabla 1.- Promedio de producción de frutos (proporción de flores que producen frutos), producción promedio de semillas por fruto y fecundidad total de los cincos de tratamientos de suplementación de polen de *Stenocereus eruca* en Estero Salinas, durante 2001 y 2002. Los valores mostrados son promedios ± 1 EE.

Tratamiento/año	Producción de frutos		Producción de semillas		Fecundidad total	
	2001	2002	2001	2002	2001	2002
1	0.00 (0.00)	0.10 (0.07)	0.00 (0.00)	14.90 (12.00)	0.00 (0.00)	1.49 (1.20)
10	0.00 (0.00)	0.23 (0.10)	0.00 (0.00)	49.00 (25.40)	0.00 (0.00)	11.32 (5.87)
100	0.55 (0.17)	0.64 (0.11)	141.09 (45.08)	170.92 (42.34)	76.95 (24.58)	109.39 (27.10)
1000	0.80 (0.13)	0.68 (0.11)	251.2 (49.32)	244.64 (46.57)	200.96 (39.46)	166.36 (31.66)
25000	0.58 (0.16)	0.56 (0.11)	191.42 (60.85)	232.48 (51.22)	111.60 (35.47)	143.90 (31.70)

fruto fueron significativas en el 2001 (Fig. 2C). No obstante que encontró una tendencia a valores más altos de fecundidad total en el tratamiento de 1000 m de distancia en el 2002, no mostró diferencias significativas con los tratamientos de 100 m y 25,000 m (Fig. 2C).

El porcentaje de germinación de semillas de los tratamientos de 1 y 10 m de distancia, no logró estimarse debido a que los frutos de estos tratamientos se encontraron parasitados y la calidad de las semillas fue baja (i.e. tamaño muy pequeño). El porcentaje de germinación fue ligeramente menor entre las semillas provenientes del tratamiento control y el de 100 m comparados con los tratamientos de 1000 y 25,000 m;, sin embargo, las diferencias entre estos tratamientos no fueron significativas (Fig. 3).

El análisis de similitud genética demostró que las cruzas realizadas entre pares de individuos a cortas distancias (1 y 10 m), eran cruzas entre ramets del mismo individuo genético (genet, Fig. 4). Los resultados de este análisis sugieren, además, que es más probable que ocurran cruzas entre parientes (endogamia biparental) cuando la polinización se da a cortas distancias, en comparación con los eventos de polinización entre pares de individuos localizados a mayores distancias (Fig. 4).

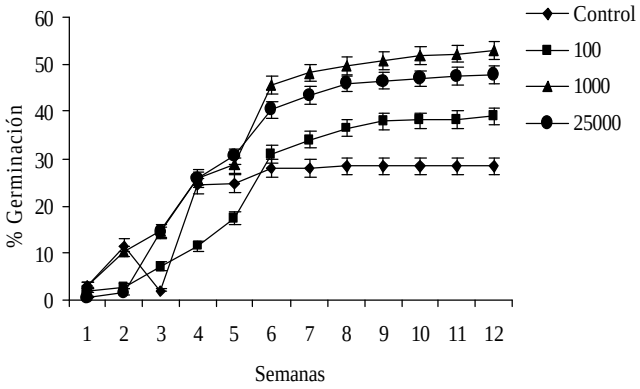

Fig. 3.- Porcentaje de germinación de los tratamientos de suplementación de polen (100, 1000 y 25000 metros) y un tratamiento control durante 12 semanas de observación. Semillas germinadas en el año 2001 Las barras representan el error estándar.

El promedio de bandas compartidas entre pares de individuos de cada tratamiento muestra rangos de 33.76± 2.83 (media ±1 DE) a 28.13±2.99 a distancias de 1 y 25,000 m, respectivamente. Los pares de plantas localizadas a 10, 100 y 1,000 m de distancia presentaron promedios intermedios 30.20±1.91, 29.70±5.34 y 30.45±4.66 porcentaje de bandas compartidas, respectivamente.

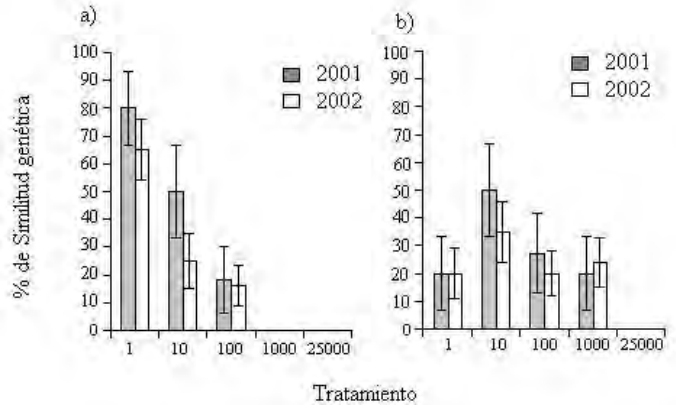

Fig. 4.- Porcentaje de similitud genética en los tratamientos de suplementación de polen durante los años 2001 y 2002, donde: a) porcentaje de geitonogamía (polinización entre flores de distintos ramets de un mismo genet) y b) porcentaje de endogamia biparental (polinización entre flores de individuos genéticamente emparentados). Las barras representas el error estándar.

Los resultados de la regresión lineal mostraron que existe una relación negativa significativa entre la distancia geográfica (en m) y la similitud genética (distancia genética), tanto en 2001 ($r^2 = 0.356$, $P < 0.001$, Fig. 5a), como en 2002 ($r^2 = 0.247$, $P < 0.001$, Fig. 5b). Asimismo, se encontró que la fecundidad total se ve afectada por la similitud genética de donadores-receptores ($r^2 = 0.353$, $P < 0.001$ y $r^2 = 0.340$, $P < 0.001$, Fig. 6ab) en ambos años de estudio, respectivamente.

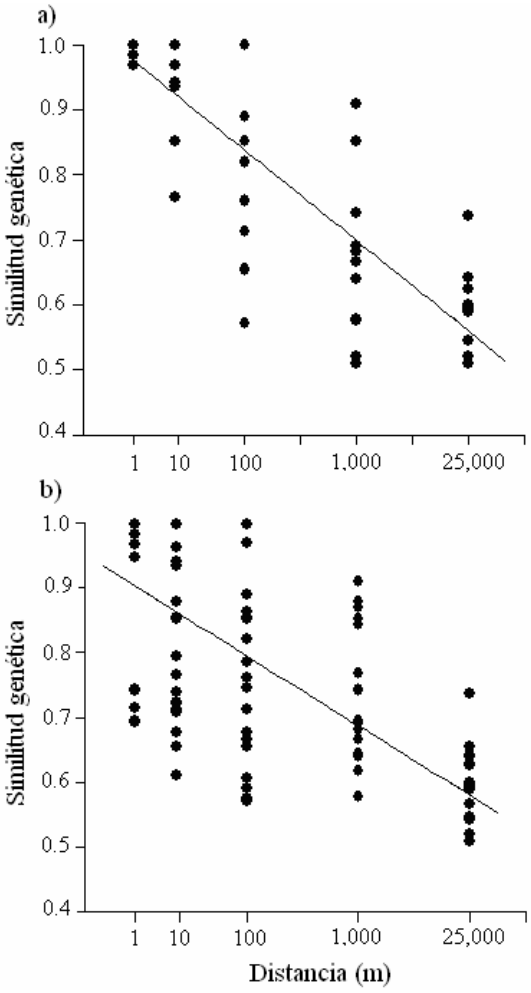

Figura 5.- Similitud genética *vs.* Distancia geográfica (m) obtenida de los tratamientos de suplementación de polen en *Stenocereus eruca* durante los años (a) 2001 ($r^2 = 0.356$, $F = 39.48$, $P < 0.001$) y (b) 2002 ($r^2 = 0.247$, $F = 35.08$, $P < 0.001$). Las líneas son el ajuste lineal.

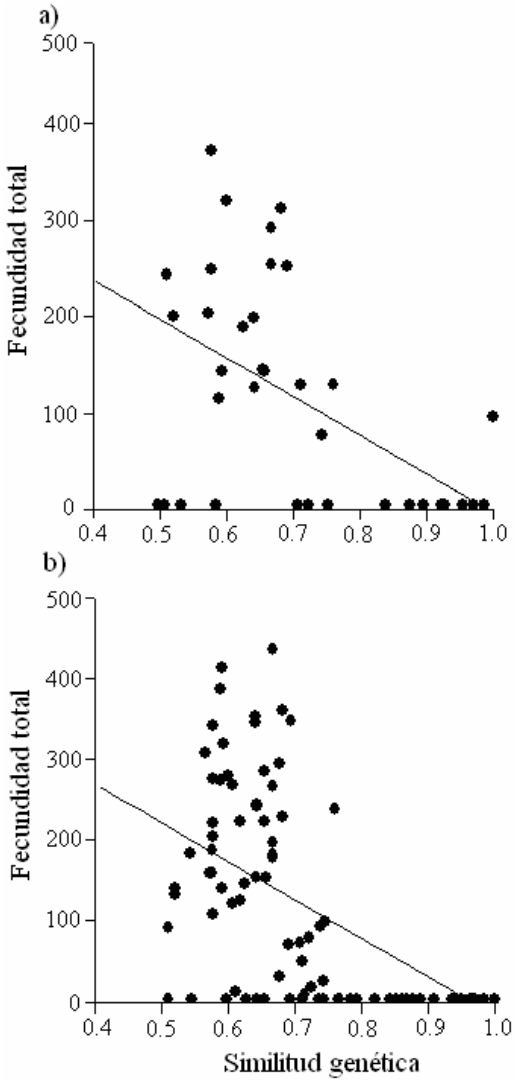

Fig. 6.- Fecundidad total por tratamiento en relación con la similitud genética entre pares de individuos de los tratamientos de suplementación de polen en *Stenocereus eruca* durante los años (a) 2001 ($r^2 = 0.353$, $F = 37.11$, $P < 0.001$) y (b) 2002 ($r^2 = 0.340$, $F = 70.01$, $P < 0.001$). Las líneas son el ajuste lineal.

Por último, el resultado de la regresión múltiple mostró que la fecundidad total de cada tratamiento de suplementación de polen se ve afectada significativamente por la distancia geográfica entre cada par de individuos (donador-receptor), así como con su respectiva similitud genética, tanto en el 2001 ($r^2 = 0.401$, $P < 0.001$, Fig. 7a), como en 2002 ($r^2 = 0.184$, $P < 0.001$, Fig. 7b).

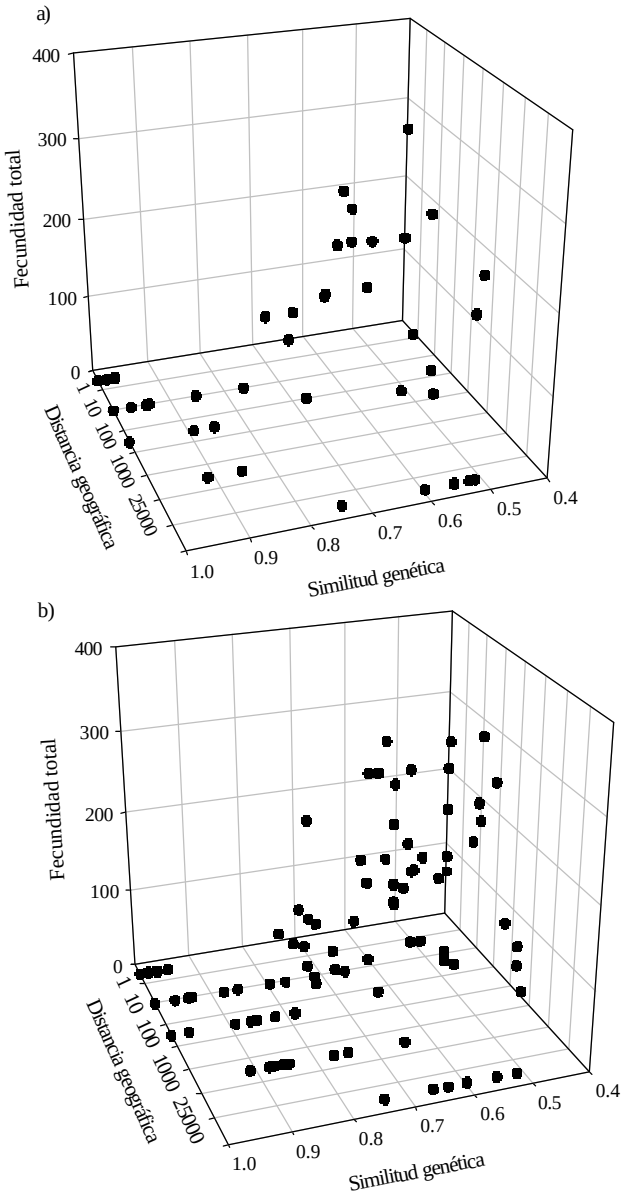

Fig. 7.- Fecundidad total por tratamiento en relación con distancia geográfica y la similitud genética entre pares de individuos de los tratamientos de suplementación de polen en *Stenocereus eruca* durante los años (a) 2001 ($r^2 = 0.421$, $P < 0.001$) y (b) 2002 ($r^2 = 0.131$, $P < 0.001$).

Discusión

En este estudio se demuestra que *Stenocereus eruca* presenta una reducción significativa de su fecundidad cuando la polinización ocurre entre individuos que se encuentran a cortas distancias, pues la similitud genética es mayor entre ramets cercanos que cuando éstos se encuentran a mayores distancias. La evidencia de los tratamientos de suplementación de polen prueban que la polinización entre flores cercanas (1 y 10 metros de distancia) experimenta altos niveles de geitonogamia y posiblemente cruzas entre parientes. Nuestros resultados no proveen evidencia sólida de que exista una distancia de entrecruzamiento óptima, ni tampoco de depresión exogámica, a pesar que aparentemente se observa una fecundidad mayor a 1000 m de distancia y una tendencia a decrecer en los tratamientos de 25,000 m, respectivamente.

Las bajas fecundidades observadas en las poblaciones de *S. eruca*, así como la ausencia de reclutamiento sexual (Capítulo 2) son similares al patrón observado en otras especies clonales (Jonsson *et al.* 1996; Charpentier *et al.* 2000; Verburg *et al.* 2000). Dicho patrón está asociado al arreglo espacial de los clones dentro de una población, el cual puede tener serias consecuencias para el movimiento de polen compatible, y consecuentemente en los niveles de entrecruzamiento y endogamia (Handel 1983, 1985; Charpentier *et al.* 2000, 2002). *Stenocereus eruca* muestra que los ramets de un mismo genet frecuentemente se encuentran agregados en el espacio, aunque también se pueden encontrar ramets de un mismo genet dispersos a lo largo de toda el área que ocupa la población (Capítulo 1). Este tipo de arreglo espacial puede ser una de las causas de la baja producción de frutos dentro de sus poblaciones (Capítulo 3), debido a que la probabilidad de geitonogamia o cruzas entre parientes situados a cortas distancias es alta durante los años cuando los polinizadores mueven el polen a cortas distancias.

Un factor determinante de la baja fecundidad de esta especie es la incertidumbre en la actividad de los polinizadores más eficientes (esfíngidos), sumada a la ineficacia de las abejas como polinizador alternativo (Capítulo 3). Las abejas son polinizadores poco eficientes de las flores de *S. eruca* ya que mueven el polen a cortas distancias, pues forrajean en flores del mismo individuo o flores cercanas a este (<10 m, Capítulo 3). Se ha sugerido que especies amenazadas, con poblaciones pequeñas presentan baja reproducción sexual debido a que son susceptibles de sufrir altos niveles de endogamia (Barret and Kohn, 1991; Byers, 1995; Sun, 1996). La evidencia obtenida en este estudio sugiere que el comportamiento de forrajeo de las abejas, en el contexto de una estructura espacial de los ramets con genotipos idénticos puede generar niveles significativos de endogamia producto de altos niveles de geitonogamia o cruzas entre parientes, lo cual ocasiona un gran desperdicio de polen. Esto puede dar lugar a una baja formación de frutos y semillas, así como a tasas reducidas de germinación de semillas, lo que ocasiona consecuentemente una disminución en la adecuación de *S. eruca*.

A pesar de que nuestros datos no revelan que exista estadísticamente una distancia óptima de entrecruzamiento en *Stenocereus eruca*, sí se observó una tendencia hacia una mayor eficiencia reproductiva (˜ 70%) entre cruzas de pares de individuos localizados a 1000 m de distancia en ambos años de estudio. Waser y Price (1989, 1991) sugieren que el grado de similitud genética entre pares de individuos puede~~n~~ afectar el éxito reproductivo de los individuos de una especie. Por ejemplo, cruzas entre individuos genéticamente similares puede incrementar los niveles de endogamia, promoviendo la aparición de efectos de depresión por endogamia en la progenie, mientras que cruzas entre individuos genéticamente muy diferentes (i.e. provenientes de diferentes poblaciones) puede originar una disminución en la adecuación producto de depresión exogámica. Nuestros resultados mostraron una baja en la eficiencia reproductiva y germinación de semillas cuando

la polinización se llevó a cabo entre pares de individuos situados a cortas distancias (1 y 10 m), así como a grandes distancias (25,000 m). Este resultado demostró una fuerte asociación entre la distancia geográfica y la similitud genética, lo cual tiene un efecto en la fecundidad de la especie. Por un lado, la polinización entre individuos situados a cortas distancias, permitió cruzas entre pares de ramets del mismo genet o entre parientes cercanos con lo que se incrementó la posibilidad de experimentar efectos por depresión endogámica y por otro, experimentar depresión exogámica a grandes distancias debido a la baja similitud genética entre pares polinizados a grandes distancias; lo cual es consistente con lo reportado en otros estudios (Waser y Price, 1989; Waser y Price, 1991; Trame *et al.* 1995).

Los costos evolutivos y ecológicos de la distribución espacial de ramets y sus consecuentes efectos sobre la frecuencia de geitonogamia pueden ser significativos, especialmente considerando las condiciones ambientales en las que esta especie se desarrolla, su limitada distribución geográfica (Gibson, 1989) y el estatus ecológico amenazado en el que se encuentra (NOM-059-ECOL-2001). En el Desierto Sonorense la variación espacio-temporal en la temperatura, así como en los eventos de precipitación tiene consecuencias significativas sobre la asignación de recursos hacia la producción de estructuras reproductivas y sobre la abundancia de polinizadores (Johnson, 1992; Fleming *et al.* 2001). Un estudio previo sugiere que *S. eruca* muestra variación espacio-temporal en el número de estructuras reproductivas y está expuesta a variaciones en la abundancia de polinizadores, lo cual limita la fecundidad femenina (Capítulo 3). Esta variación, en combinación con altos niveles de geitonogamia y cruzas entre parientes, pueden ser responsables de que los reclutamientos de plántulas originadas de semillas en las poblaciones de *S. eruca* estén restringidos a "ventanas de oportunidad" (Jerling y Cheliak, 1992) o a pulsos asociados a sucesos climáticos, lo cual es característico de otras especies suculentas (Jordan y Nobel, 1979; Turner, 1990; Pierson y Turner, 1998). Se ha sugerido que especies raras o endémicas con poblaciones fragmentadas son más susceptibles a los efectos de factores reproductivos, demográficos y genéticos lo cual las convierte, en especies potencialmente más vulnerables a la extinción (Lande, 1988; Barret and Kohn, 1991; Ellstrand y Ellam, 1993; Schemske *et al.* 1994; Byers, 1995; Kephart *et al*., 2002; Oostermeijer *et al*. 2003). No obstante que evidencia genética y demográfica sugieren que *S. eruca* no se encuentra en peligro de extinción, debido a que presenta niveles considerables de variación genética y tasas de crecimiento poblacional estables (Capítulo 1 y 2), los efectos de la estructura clonal sobre el sistema reproductivo pudieran estar generando altos niveles de geitonogamia, y cruzas entre parientes, así como estar restringiendo la existencia de una distancia óptima de entrecruzamiento. Esto pudiera estar generando que las poblaciones de *S. eruca* exhiban altos niveles de endogamia, tal como lo sugiere un estudio con isoenzimas (*Fis* = 0.781; Clark-Tapia, 2000), lo cual sumado al estatus endémico y alto grado de fragmentación a que están sujetas las poblaciones de *S. eruca*, puede causar a futuro una reducción en los niveles de variación genética, pérdida de riqueza alélica y ocasionar que la especie sea más susceptible a la extinción. Es necesario por ello, estudios que evalúen los efectos de depresión endogámica con la finalidad de conocer los efectos reales que ocasionan altos niveles de geitonogamia y cruzas entre parientes que permitirán conocer de una manera más detallada la influencia de la estructura clonal y proponer con ello estrategias de conservación viables en esta especie.

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DISCUSION GENERAL

Reproducción sexual *vs*. Propagación clonal

Los resultados obtenidos en este estudio, al integrar una combinación de herramientas genéticas y demográficas, muestran un patrón contrastante en relación con la importancia relativa del reclutamiento sexual *vs*. clonal en las poblaciones de *Stenocereus eruca*. Por un lado, el análisis genético realizado con RAPDS demuestra que las poblaciones de *S. eruca* son multi-clonales, con altos niveles de diversidad genotípica (Capítulo 1). Este resultado sugiere que el reclutamiento sexual es un mecanismo importante en la regeneración en las poblaciones de esta especie. Esta evidencia genética, sin embargo, contradice las observaciones de campo (Gibson, 1989; Turner *et al.*, 1995; Clark-Tapia, 2000) y los resultados del estudio demográfico (Capítulo 2), que muestran una ausencia de reclutamiento sexual en los años de estudio (1999-2002) y una contribución mucho mayor de la propagación clonal como mecanismo de regeneración de las poblaciones de esta especie. La ausencia de reclutamiento sexual observada en el campo y reflejada en los resultados del análisis demográfico es consistente con los datos reproductivos (Capítulo 3), que sugieren una baja fecundidad producto de una producción limitada de frutos en algunos sitios, en los años de estudio. Lo anterior parece ser consecuencia de la variación temporal en la abundancia de polinizadores (esfíngidos), o posiblemente de un incremento en los eventos de geitonogamia ocasionado por el arreglo espacial que presentan los clones dentro de las poblaciones.

A pesar de que *S. eruca* produce consistentemente semillas viables (Capítulo 3), no se ha observado reclutamiento de plántulas (*e.g*. vía sexual) en sus poblaciones en más de una década (c.a.15 años, Gibson, 1989; Turner *et al.*, 1995; Clark-Tapia, 2000). Esta evidencia coincide con la obtenida en otras especies clonales (Jordan y Nobel, 1979; Cheliak y Pitel, 1984; Johnson *et al.*, 1996; Verburg *et al.*, 2000; Hangelbroek *et al.*, 2002), en las que la reproducción sexual se ve limitada por factores densodependientes y densoindependientes. En

S. eruca los factores densodependientes (alta depredación de semillas, variación temporal o escasez de polinizadores) y densoindependientes (altos niveles estrés hídrico o altas temperaturas) reducen el número de estructuras reproductivas y semillas, limitando consecuentemente los eventos de reproducción sexual, que se dan de manera esporádica (ver Capítulos 2 y 3).

El hecho de que los eventos de reproducción sexual estén restringidos a "ventanas de oportunidad o pulsos" (Jeliski y Cheliak, 1992) asociadas a sucesos climáticos particulares, es común en especies suculentas (Jordan y Nobel, 1979; Turner, 1990; Pierson y Turner, 1998) y puede ser la razón por la cual *S. eruca* se ha considerado como una especie predominantemente clonal, y como un caso extremo de clonalidad dentro de las cactáceas (Gibson y Nobel 1986). Sin embargo, este trabajo muestra que existen contradicciones entre las evidencias genéticas y las demográfico-reproductivas a este respecto, lo que reafirma la importancia de utilizar una combinación de herramientas para la evaluación de un aspecto determinado de la biología de las especies vegetales porque podemos caer fácilmente en errores de apreciación al no considerar la diversidad de escalas temporales de los diferentes enfoques de estudio. En este caso el análisis demográfico-reproductivo sugiere que el mecanismo de regeneración más importante es la propagación vegetativa. En cambio, el análisis genético sugiere, con base en los altos valores de diversidad clonal y arreglo espacial de los clones, que el reclutamiento sexual quizá de forma esporádica esta asociado a "ventanas de oportunidad" que ocurren de manera irregular tiene una contribución importante al mantenimiento de la población, similar al de propagación clonal. Obviamente, la escala temporal en la que ocurren dichas oportunidades de reclutamiento por vía sexual debe ser considerablemente extensa (i.e. décadas o centurias, Jordan y Nobel, 1979; Jeliski y Cheliak, 1992), por lo que en una escala de tiempo más limitada es improbable observar dichos fenómenos**.** En este sentido este estudio demuestra una limitación del método demográfico en

lo que respecta a su poder de inferencia de la importancia de la reproducción sexual en el tiempo ecológico y resalta la importancia de incorporar el enfoque genético-poblacional, el cual ayuda a entender procesos que ocurren a escalas temporales difíciles de entender en estudios ecológicos.

Demografía de genets y ramets

La dificultad de identificar genets en el campo es una de las grandes limitantes para realizar estudios demográficos a este nivel en especies de plantas clonales. En *S. eruca*, la fragmentación de los tallos dificulta la distinción de genets en el campo. Sin embargo, el análisis de la estructura clonal espacial a base de RAPDS (Capítulo 1) permitió conocer la identidad genética de cada ramet y definir la extensión espacial de los genets en la población de Estero Salinas. Esto proporcionó herramientas para comprender mejor la dinámica poblacional a nivel de genets y ramets y evaluar el papel que juegan la reproducción sexual y la propagación clonal en la dinámica de esta población. Debe mencionarse que el análisis espacial de clones se llevó a cabo sólo en una población (n = 282) a causa de limitaciones prácticas.

La alta incidencia de propagación clonal detectada en *S. eruca* a través del análisis demográfico, parece operar como una estrategia dirigida a mantener y estabilizar la población donde *S. eruca* se desarrolla. Esta forma de crecimiento y de ocupación del espacio que permite el mantenimiento de las poblaciones durante periodos de baja reproducción sexual es característica de una gran variedad de plantas clonales, que habitan ambientes relativamente impredecibles, como los desiertos (Muller, 1953; Parker y Hamrick, 1992; Mandujano *et al.*, 2001). Aunque la evidencia molecular sugiera que ambas formas de propagación (sexual y clonal) son importantes en el mantenimiento de las poblaciones de *S. eruca*, los resultados del análisis demográfico indican que la aportación clonal (a través de la producción de ramets) es significativamente más importante para el crecimiento poblacional que la reproducción sexual

(Capítulo 2). La capacidad ilimitada de crecimiento, las bajas tasas de mortalidad y los costos aparentemente bajos del establecimiento de los ramets, permiten que un individuo de esta especie alcance rápidamente una clase de tamaño particular en el ciclo de vida, permitiendo la colonización de espacios e incrementando el número de individuos reproductivos dentro de una población. Estas características, comunes a otras especies clonales (De Steven, 1989; Mendoza, 1994; Zuidema, 2000; Mandujano *et al.*, 2001), pueden ser la causa de la mayor importancia relativa de la propagación clonal en el crecimiento poblacional detectada en el análisis demográfico.

El análisis de la demografía de esta especie a nivel de genets indica que la propagación clonal representa un proceso de crecimiento y ocupación del espacio de los genets más que una forma de reproducción, el cual a su vez permite evitar la senescencia e incrementar la adecuación de los genets (De Steven, 1989; Zuidema, 2000). En contraste con la baja sobreveviencia de plántulas (0%) atribuible a diversos factores (i.e. depredación, altas temperaturas), la sobrevivencia a nivel de genet es del 100%. Un genet puede ser prácticamente inmortal a través de la producción de ramets (Eriksson and Jerling, 1990). La evidencia genético-demográfica sugiere que un genet de *S. eruca* puede tener una longevidad mayor a 1000 años (Capítulo 2). Sin embargo, es necesario tomar con cautela estos resultados dado el corto periodo de tiempo empleado para estimar la edad.

Consecuencias evolutivas de la reproducción sexual y de la propagación clonal en *Stenocereus eruca*

El balance entre las dos formas de reclutamiento aquí discutidas (sexual *vs*. clonal) puede representar una característica adaptativa de *S. eruca*, que asegura su persistencia por largos periodos de tiempo manteniendo a la vez altos niveles de diversidad genética a partir del reclutamiento de plántulas por vía sexual de manera esporádica (Capítulo 1 y 2) en ambientes impredecibles tales como los desiertos.

A pesar que la producción de ramet vía propagación clonal en *S. eruca* puede ser una adecuada estrategia que le permite sobrevivir en condiciones extremas, ésta puede tener serias consecuencias ecológicas y evolutivas en la dinámica de genet de *S. eruca*. Por un lado, la dinámica de genets puede verse afectada en la generación de nuevos individuos (i.e. la producción de semillas o de ramets) debido a una asignación de recursos desigual entre la reproducción sexual y propagación clonal (compromisos fisiológicos) y con ello favorecer a una de las dos estrategias reproductivas (Abrahamson, 1980; Cheplick, 1995; Mendoza y Franco, 1995; Prati y Schmid, 2000) y por otro, incrementar la probabilidad de eventos de geitonogamia y cruzas entre parientes (Handel, 1985; Trame *et al.* 1995; Charpentier *et al.*, 2000, Eckert, 2000; Charpentier, 2002). Aunque no se han realizado estudios acerca de la asignación de recursos hacia la reproducción sexual vs clonal en *S. eruca*, las observaciones de campo y evidencias demográficas (capitulo 2) sugieren que la producción de ramets recibe mayor asignación de recursos que la reproducción sexual. Asimismo, en este sentido los datos a nivel de genets sugieren que *S. eruca* invierte más a su crecimiento que a producir estructuras reproductivas.

Por otro lado, la capacidad de un genet de producir un número ilimitado de ramets puede incrementar potencialmente su adecuación, debido a que cada ramet tiene la capacidad de reproducirse y dar lugar a nuevos genets. Sin embargo, el arreglo o distribución espacial de estos ramets en una población puede tener serias implicaciones en la función y mantenimiento de la reproducción sexual, debido a que el crecimiento clonal puede interferir con los patrones de polinización y el sistema reproductivo (Handel, 1985 y Charpentier *et al.*, 2000). *Stenocereus eruca*, muestra una distribución espacial predominantemente agregada, aunque también se detectaron genets dispersos a lo largo de toda la población (Capítulo 1). Este arreglo espacial de ramets en combinación con la auto-incompatibilidad de la especie puede ser una de las causas de la baja producción de frutos (Capítulo 3a), debido a que puede

provocar una alta incidencia de eventos de geitonogamia que probablemente conducen a reducir la fecundidad y a incrementar los riesgos de depresión por endogamia tal como se infiere a partir de los resultados del estudio de suplementación de polen que se reporta en el Capítulo 3b. Dichos resultados sugieren que si el flujo de polen es espacialmente limitado, se generaría un gran desperdicio de polen y una disminución en la adecuación de *S. eruca*, por lo menos a distancias menores de 10 m. Evidencia obtenida con isoenzimas (Clark-Tapia, 2000) sugieren niveles significativos de endogamia para la especie. En este sentido, la variación genética espacial estructurada dentro de las poblaciones debido a la propagación vegetativa y el comportamiento de forrajeo de las abejas en el contexto de una estructura espacial de los ramets con genotipos idénticos tienen un costo para la especie, ya que pueden promover niveles considerables de endogamia, lo cual da lugar a una baja fecundidad (producción de frutos y semillas), así como a tasas reducidas de germinación de semillas, que pueden agravar mas aun el problema del bajo reclutamiento sexual en las poblaciones de *S. eruca* (Capítulo 3ab).

Este estudio demuestra que la baja fecundidad en *Stenocereus eruca* (parámetro demográfico básico en la regeneración de sus poblaciones) es producto de la interacción de factores genéticos (organización espacial de genotipos dentro de la poblaciones y sistema reproductivo) y de factores ecológicos (abundancia y comportamiento forrajero de los polinizadores). Ejemplo de ello se mencionó en el párrafo anterior, sin embargo otro ejemplo que es producto de esta interacción es la calidad de polen intercambiada entre pares de individuos (donador y receptor de polen) durante un evento de post-polinización (Waser *et al.*, 1987; Waser y Price, 1989; Waser y Price, 1991; Trame *et al.* 1995). En este sentido, el factor genético y movimiento de los polinizadores pueden generar que las distancias de entrecruzamiento afecten la calidad de polen (producto de la polinización entre pares de individuos genéticamente similares; Capítulo 3b), lo cual puede tener por un lado un efecto

adicional en la fecundidad de *S. eruca*, y por otro lado experimentar efectos por depresión endogámica debido a cruzas entre parientes.

La estrategia evolutiva de historia de vida de *S. eruca*, de apostar a lo seguro (bet-hedging, Stearns, 1976) por medio de la producción de ramet vía propagación clonal contra cualquier falla reproductiva, está probablemente creando un conflicto evolutivo con el sistema reproductivo de está especie. Por ejemplo, al crecer el genet por medio de la producción de ramets se incrementa la probabilidad de cruzas entre ramets del mismo genet (geitonogamia) o entre parientes en consecuencia la reproducción sexual se iría seleccionando en contra. En especies que no presentan recombinación genética, tales como las clonales, esto puede incrementar con el tiempo una acumulación de mutaciones somáticas con lo cual se limita aún más las cruzas entre parientes (i.e. debido a un aumento en la carga genética y a depresión por endogamia), de manera que la adecuación de la especie disminuirá cada vez más con el tiempo hasta que se extingue (estilo "engrane" de Muller, Muller 1964; Felsenstein, 1974). Este conflicto clonal-reproductivo no es exclusivo de *S. eruca*, dado que está documentado en otras especies (Handel, 1985; Trame et al. 1995; Charpentier et al. 2000), por lo que habrá de reconsiderar si los beneficios ecológicos que aporta la clonalidad (i.e. estabilidad de poblaciones o permanencia por largos periodos de tiempo) son realmente una ventaja evolutiva a largo plazo para las especies clonales.

Estrategias de Conservación

La integración de diversas herramientas (genéticas, demográficas y de biología reproductiva), como las utilizadas en este estudio, es relevante en el diseño de estrategias de conservación para las especies de plantas vulnerables y amenazadas. A pesar de que la integración de estas herramientas es de interés, tanto para biólogos evolutivos como conservacionistas, son muy escasos los estudios que involucren aspectos genéticos-demográficos-reproductivos (i.e. Jain, 1994; Trame *et al.*, 1995; Goodell *et al.*, 1997; Young

et al., 2002). Esto se debe principalmente a conflictos de enfoque y a la tendencia a atribuirle una mayor importancia a la evaluación de aspectos genéticos y/o demográficos en especies raras o amenazadas (Lande 1988; Schemske *et al*., 1994).

La integración de diversas herramientas en este estudio permitió evaluar el estado actual de las poblaciones de *S. eruca*. La evidencia obtenida en este trabajo sugiere que las poblaciones de esta especie no se encuentran en peligro de extinción desde una perspectiva genético-demográfica o de biología reproductiva, por lo menos en las cuatro poblaciones estudiadas. Esto debido por un lado, a que los valores de variabilidad genética encontrados en las cuatro poblaciones de *S. eruca* son relativamente altos comparados a otras especies raras o amenazadas (i.e. Torres *et al*., 2003) o semejantes a otras especies analizadas con RAPDs (Nybom and Bartish, 2000; Sánchez, 2002; Navarro *et al.* 2003), y por otro, a que el análisis demográfico sugiere que las poblaciones de *S. eruca* se encuentran en equilibrio demográfico y, por lo menos dentro de las áreas estudiadas, no se encontraron vestigios de perturbación antropogénica o de colección ilegal de ejemplares.

A pesar de que las evidencias anteriores son satisfactorias considerando que *S. eruca* es una especie endémica amenazada, la síntesis entre estos tres métodos (genético-demográfico- reproductivo) sugiere un escenario a futuro no muy prometedor para esta especie. Diversos estudios han documentado que especies endémicas o restringidas y sujetas a severos disturbios antropogénicos y con bajos niveles de reproducción sexual, son potencialmente más vulnerables a la extinción que especies con amplia distribución (Drury, 1980; Byers, 1995; Oostermeijer *et al.*, 2003) debido a que son propensas a presentar altos niveles de endogamia, flujo génico reducido y una alta diferenciación genética entre poblaciones (Barret and Kohn, 1991; Templento *et al*., 1990, Frankham, 1995). En este sentido, dentro de las poblaciones de *S. eruca* hemos detectado severos disturbios antropogénicos en áreas cercanas a los sitios estudiados producto del cambio de uso de suelo

(agricultura) o de actividades asociadas a asentamientos humanos (quema de plantas, acumulación de basura). Estas actividades han aumentado en frecuencia y están fragmentando las poblaciones, lo cual aunado a la escasa apreciación hacia esta especie por parte de las comunidades locales, pone en riesgo la persistencia de *S. eruca*. Asimismo, sumado a esto la estructura genética espacial observada en una población (producto de flujo génico limitado y propagación clonal) y los niveles de endogamia posiblemente producto de cruzas entre parientes puede tener a futuro efectos potenciales en la estructura genética y en el comportamiento demográfico de esta especie poniendo en riesgo la viabilidad de sus poblaciones. Por este motivo considero que una estrategia viable de conservación para esta especie sería la creación de una reserva estatal o federal en Estero Salinas. Esta zona resulta ideal debido a su bajo nivel de perturbación y por estar realmente alejada y por lo tanto aislada de zonas urbanas. Además, este sitio presenta niveles considerables de variación genética y es la única población donde a pesar de la baja producción de frutos, regularmente se ha observado la reproducción sexual (Capítulo 1 y 3). Finalmente, es muy importante implementar programas de educación ambiental para fomentar la apreciación y el conocimiento sobre la importancia biológica de *S. eruca* entre los pobladores de la región y propiciar la cooperación de la gente en su conservación.

CONCLUSION

En este trabajo se describe la estructura clonal, la demografía y la biología reproductiva de *Stenocereus eruca*. El estudio demográfico demostró que el reclutamiento por la vía clonal fue mas importante en el mantenimiento de las poblaciones. El estudio de la biología reproductiva mostró que la eficiencia reproductiva es baja probablemente debido a limitación por polinizadores. Sin embargo, el estudio de la estructura clonal probó que las poblaciones son multiclonales y que le reproducción sexual parece ser importante. Estas aparentes contradicciones parecen ser resultado de las diferentes escalas de tiempo que son abordadas por los diferentes enfoques de estudio. Este estudio mostró una limitación del método demográfico y resalta la importancia de incorporar diferentes herramientas de estudio, lo cual permite explorar y entender aspectos ecológicos imposibles de evaluar a corto plazo, sobre todo si consideramos que *Stenocereus eruca*, es una especie que presenta reclutamientos asociados a "ventanas de oportunidad" que ocurren de manera muy esporádica. En este sentido, el enfoque genético permitió también evaluar la demográfica de genets en una población (Estero Salinas), a través de la identificación genética de cada individuo y además evaluar la identidad genética de donador y receptor de polen utilizados en el experimento de suplementación de polen. Lo anterior permitió conocer por un lado que la propagación clonal representa un proceso de crecimiento y ocupación del espacio de los genets, más que una forma de reproducción, y que a su vez permite incrementar la adecuación de los genets, y por otro lado, conocer que a pesar que la propagación clonal en *S. eruca* puede ser una estrategia apropiada que le permite sobrevivir en condiciones extremas, ésta puede tener serias consecuencias ecológicas y evolutivas en la dinámica de genet de *S. eruca* al tener efectos en el sistema reproductivo.

A pesar que faltaron problemas importantes por abordar como estimar las tasas de crecimiento (λ) de genotipos y tasas de entrecruzamiento en semillas, este trabajo permite

vislumbrar una síntesis entre estos tres enfoques (genético-demográfico-reproductivo) al evaluar la importancia relativa de dos parámetros demográficos importantes (fecundidad y propagación clonal), lo cual permitió reconsiderar si los beneficios ecológicos que aporta la clonalidad son realmente una ventaja evolutiva a largo plazo para las especies clonales y permitió además conocer la situación ecológica actual de esta especie para proponer una estrategia viable de conservación.

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