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EFECTO DE LA DENSIDAD POBLACIONAL EN LA DINAMICA DEMOGRAFICA, ECOLOGIA REPRODUCTIVA Y ESTRUCTURA GENETICA DE *Flourensia cernua* D C.

TESIS

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Resumen

Flourensia cernua es un arbusto del Desierto Chihuahuense que florece en invierno, tiene un síndrome de polinización anemófilo y se presenta tanto en matorrales con densidad poblacional alta (matorrales densos), como en matorrales con densidad baja (matorrales difusos).

Este trabajo tuvo como objetivo estudiar el efecto de la densidad poblacional en la adecuación femenina de los individuos, en el sistema reproductivo y en la estructura genética de la población. Teniendo en cuenta que la disponibilidad de polen exógeno aumenta con la densidad poblacional en plantas anemófilas, se espera que tanto la adecuación femenina como las tasas de polinización cruzada se incrementen con la densidad. Por otro lado, dado que la diferenciación genética entre poblaciones aumenta con la tasa de polinización cruzada, se espera una mayor diversidad genética en las poblaciones densas que en las difusas y diferenciación genética entre ambas.

Los resultados mostraron que la densidad poblacional de *F. cernua* afecta dos características de la biología reproductiva de esta especie. En los matorrales difusos hay una mayor depredación de flores y frutos y mayor limitación por la cantidad de polen. Los matorrales densos y difusos tienen un bajo potencial reproductivo (*i. e.* 0 a 22 semillas viables por individuo). Los factores limitantes de la fecundidad de *F. cernua* son: la aborción de frutos (90%), la baja viabilidad de semillas (menor al 10%), los fallos en polinización (25% de las flores no depredadas) y la depredación pre-dispersión de flores y frutos (25% de las flores iniciales).

Tanto la expresión de la auto-incompatibilidad (45% de los individuos son capaces de autopolinizarse), como la depresión endogámica en la viabilidad de las semillas ($\delta = -1.25$ a $\delta = 1$) presentan una gran variación entre individuos, pero no entre tipos de matorral. La diversidad genética de la especie es alta ($H_o = 0.41$ a 0.59) y existe un exceso de hetérocigos ($F_{IS} = -0.15$). Los matorrales densos y difusos no difieren ni en sus frecuencias alélicas $F_{ST} = -0.013$, ni en sus tasas de entrecruzamiento ($t_m = 1$)

Flourensia cernua se caracteriza por ser parcialmente auto-incompatible, lo que le permite mantener un sistema de apareamiento mixto. Esta característica influye de manera preponderante en la adecuación de los individuos, la diversidad y estructura genética de las poblaciones, sobrepasando el efecto que tiene la densidad poblacional y explica parcialmente la similitud entre los matorrales densos y difusos.

Los resultados también sugieren que la selección natural puede favorecer a los individuos que tengan una mayor sincronía floral con el resto de la población, a los que tengan una auto-compatibilidad mayor y a los individuos heterócigos. Planteamos además una hipótesis sobre los factores ecológicos y genéticos que pueden influir en la evolución del sistema de auto-incompatibilidad (e. g. cambios en los vectores de polinización, limitación por polen compatible y baja diversidad de alelos de auto-incompatibilidad), para explorarse en investigaciones futuras.

Abstract

Flourensia cernua is a Chihuahuan Desert shrub. It flowers in winter and has an anemophyllous pollination syndrome. The species is found in scrubs with high density (dense scrub) and in scrubs with low density (diffuse scrub).

In this paper the effect of population density in the female fitness of plants, in the breeding system and genetic structure of population is analyzed. We expect that the female fitness and the outcrossing rates increase as population density does, due to a larger availability of pollen. Additionally we expect to found a higher genetic diversity in dense scrubs, and genetic differentiation between scrub types.

The population density had an effect in two traits of *F. cernua* reproductive biology. First, in diffuse scrubs a higher flower and fruit predation was found and second, in this scrub the pollen limitation is higher than in dense scrub. Both scrub types had a lower reproductive potential (*i. e.* 0 to 22 viable seeds per plant). The four main processes that limit the reproductive potential were: a) predation by beetles (ca. 25% of initial flowers), b) pollination failures (ca. 25% of non predated flowers), c) abortion (ca. 90% of initiated fruits) and d) low germinability rates (<10% seeds germinated).

We found a high variability among plants in the degree of self-incompatibility (45% of individuals could produce seeds after selfing), and in the inbreeding depression in the seed germinability ($\delta = -1.25$ to $\delta = 1$), but not variability between scrub types. Genetic diversity was high ($H_o = 0.41$ to 0.59) and an heterozygous excess was found ($F_{IS} = -0.15$). There was not genetic differentiation between scrub types ($F_{ST} = -0.013$) and similar outcrossing rates wer found in both scrub types ($t_m = 1$).

Flourensia cernua is a partially self-incompatible species and has a mixed mating system. This trait had an important effect in the female fitness and in the genetic structure and diversity. This effect was even larger than the one that population density had and partially explain the similarities between scrub types.

We suggest that higher flowering synchrony, higher self-compatibility and higher heterozygosity could be favor by natural selection in *F. cernua*. We propose that ecological and genetic factors are influencing the evolution of self-incompatibility system (e. g. shifts in pollination vectors, low availability of compatible pollen and los S-allele diversity). These hypothesis will be explored in future research.

In memoriam
Guillermo Ferrer Veraza
1936-1999

"Something lay in the shadow at the foot of the ridge, as stiff as the stick of the fallen rocket; and the man who knew too much knew what is worth knowing."
Gilbert K. Chesterton. "The man who knew too much."

A Mamá, Gabuchito, Tía Anita, Odette y Grillo
"On ne voit bien qu'avec le cour. L'essentiel est invisible pour les yeux."
Antoine de Saint-Exupery ."Le Petit Prince"

A mis amigos
"Mais, si tu m'apprivoises, nous aurons besoin l'un de l'autre. Tu seras pour moi unique au monde. Je serai pour toi unique au monde..."
Antoine de Saint-Exupery ."Le Petit Prince"

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Capitulado de tesis

Efecto de la densidad poblacional en la dinámica demográfica, ecología reproductiva y estructura genética de *Flourensia cernua* D C.

Miriam Monserrat Ferrer Ortega

INTRODUCCIÓN

Una población es el conjunto de individuos de una misma especie que habita en una misma área. Este grupo de individuos puede caracterizarse por su estructura genética, estructura espacial, estructura de edades y estructura de tamaños (Harper, 1977; Silvertown y Lovett-Doust, 1993). La biología de poblaciones estudia los cambios en la estructura genética de las poblaciones a través del tiempo (*i. e.*, evolución) y los cambios en la estructura espacial, de edades y/o de tamaños de las poblaciones (*i. e.*, dinámica demográfica) (Silvertown y Lovett-Doust, 1993). Los estudios de biología de poblaciones de una especie son, por lo tanto, básicos en el conocimiento de la trayectoria evolutiva de la misma (Wright, 1965; Futuyma, 1998) y han cobrado gran importancia en la biología de la conservación (Boyce, 1992; Menges, 2000).

Los análisis comparativos en poblaciones vegetales que han incluido tanto información demográfica como del sistema reproductivo y de la genética de poblaciones naturales son el sustento de estudios clásicos relacionados con la evolución de los sistemas reproductivos (Schemske y Lande, 1985; Charlesworth y Charlesworth, 1987; Husband y Schemske, 1996) y la evolución de las historias de vida (Stearns, 1992; Silvertown y Franco, 1996). En un contexto ecológico, los estudios comparativos permiten identificar los patrones de variación espacial y temporal en la expresión de los sistemas reproductivos (Levin, 1988; Levin, 1996), los patrones de variación genética (Hamrick y Godt, 1990; Hamrick y Godt; 1996) y los factores que limitan la adecuación de los individuos (Sutherland y Delph, 1984; Lovett-Doust y Lovett Doust, 1992; Burd, 1994; Richards, 1996; Larson y Barret, 2000). A pesar de la importancia que estos trabajos integrales revisten son pocos los estudios que se han desarrollado en poblaciones de plantas perennes. Entre ellos se encuentran los realizados con *Pinus ponderosa* (Linhart, *et al.*, 1981), *Astrocaryum mexicanum* (Eguiarte, 1990), *Cecropia obtusifolia* (Alvarez-Buylla, *et al.*, 1996), *Prosopis glandulosa* var. *torreyana* (Gulobov *et al.*, 1999; Gulobov *et al.*, 2000) y en *Opuntia* *rastrera* (Mandujano *et al.*, 1996; Mandujano *et al.*, 2002).

El trabajo que se presenta en esta tesis doctoral intenta aportar una visión de algunos aspectos de la biología de poblaciones de *Flourensia cernua* D C. (Asteraceae), un arbusto característico del Desierto Chihuahuense.Esta especie crece en dos tipos de matorral que contrastan en su densidad poblacional. El trabajo se dividió en tres capítulos que comprenden: 1) La variación temporal y espacial en la adecuación femenina y la fecundidad potencial en diferentes poblaciones y el efecto que tiene la estructuración reproductiva (definida por la densidad de plantas y la sincronía de la floración) en la adecuación femenina. 2) La expresión del sistema de auto-incompatibilidad y el efecto de la depresión endogámica en la adecuación femenina en estos dos ambientes; y 3) La variación en las tasas de polinización cruzada y su efecto en la estructura genética.

En este primer capítulo introductorio se presenta una revisión bibliográfica sobre los sistemas de auto-incompatibilidad y la diversidad genética y estructura genética en la familiaAsteraceae. Se presenta también una descripción extensa de *Flourensia cernua* y del sitio de estudio, tanto de las formaciones vegetales, como de las características climáticas.

Sistemas de auto incompatibilidad en Asteraceae

La auto-incompatibilidad (self-incompatibility) se define como la falta de capacidad de una planta hermafrodita fértil para producir frutos después de ser auto-polinizada (Heslop-Harrison, 1975; de Nettancourt, 1977; Husband, 1988). Los sistemas de auto-incompatibilidad genética impiden la auto-fertilización, aún a riesgo de una baja producción de semillas (Richards, 1986). En las flores la reacción de auto-incompatibilidad se puede detectar en la superficie estigmática, cuando los granos de polen del mismo individuo no se adhieren, o cuando aún adheridos no presentan germinación o no logran penetrar la superficie estigmática y dentro del estilo cuando el crecimiento de los tubos polínicos se impide y al desintegrarse los tubos se forman cuerpos callosos (Heslop-Harrison, 1975; Figura 1).

La familia Asteraceae se caracteriza por la presencia de un sistema genético de auto-incompatibilidad esporofítica multialélica (de Nettancourt, 1977; Richards, 1996). Este sistema de incompatibilidad está codificado por varios alelos (S) de un único locus, y está relacionado con la expresión de proteínas de la pared del grano de polen (Howlett *et al.*, 1975). Si el polen y el óvulo comparten un alelo S de auto-incompatibilidad, la reacción de incompatibilidad se inicia e impide la auto-polinización (Gerstel, 1950). El mecanismo de incompatibilidad que se presenta en la familia Asteraceae fue descrito desde la década de los 50's por Gerstel (1950) utilizando como modelo a *Parthenium argentatum,* por Hughes y Babcok (1950) con *Crepis foetidus,* y por Crowe (1954) en *Cosmos bipinnatus*. Desde esos estudios pioneros se han reportado al menos 30 géneros de asteraceas auto-incompatibles (*e. g.* Davis, 1986; Brauner y Gottlieb, 1987; Campplitt, 1987; Gross y Werner, 1983; Watson y Estes, 1988; Brauner *et al.,* 1988; Dierenger y Cabrera, 1989; Widén, 1991; Buchloe *et al*., 1992; Mejías, 1994; Byers, 1995; Gavrilova, 1996; Giblin y Hamilton, 1999).

La determinación de la presencia del sistema de auto-incompatibilidad en Asteraceae se ha derivado de trabajos de polinización, así como de observaciones microscópicas en las que se identifica la reacción de auto-incompatibilidad y a la fecha se cuenta al menos con registros para 226 especies de la familia. Con el fin de explorar la existencia de patrones en la expresión de la auto-incompatibilidad asociados con la forma de vida y los ambientes ecológicos, se presenta a continuación un análisis gráfico de los resultados de los estudios para estas 226 especies. Para ello, las características de las especies se agruparon en tres categorías de auto-incompatibilidad: 1) auto-incompatibles (la producción de semillas después de la auto-polinización es nula), 2) parcialmente auto-compatibles (la producción de semillas después de la auto-polinización es significativamente menor que la producción de semillas a partir de polinización cruzada, seudo-auto fertilizadas *sensu* Levin, 1996) y 3) auto-compatibles (producción de semillas a partir de auto-polinización igual o mayor a la de polinización cruzada).

Patrones por forma de vida

Se ha propuesto que el ancestro de las especies de la familia Asteraceae es una planta auto-incompatible estricta y que la auto-compatibilidad es un rasgo apomórfico asociado con especies anuales (Stebbins, 1974; Richards, 1996; Lane, 1996). De las 226 especies analizadas, el 66% se caracterizan como auto-incompatibles, un 10% son parcialmente auto-compatibles y el 24% son auto-compatibles. La gran proporción de especies que son auto-incompatibles y parcialmente auto-compatibles (76%) nos indican que la presencia del sistema de auto-incompatibilidad es característica de Asteraceae, pero que la expresión de este sistema es variable en al menos un 10% de las especies, lo que puede estar asociado con características como la forma de vida(hábito de crecimiento) y la duración del ciclo de vida.

El análisis de las 226 especies de asteraceas agrupadas por forma de vida (árboles, arbustos y herbáceas –estas últimas se dividieron en cuatro categorías, de acuerdo al ciclo de vida de la especie; *i. e.* anual, bianual o perenne y aquellas que pueden presentar tanto ciclos de vida anuales como perennes-) nos indica que la presencia del rompimiento parcial en el sistema de auto-incompatibilidad está asociado con las formas herbáceas y que dentro de éstas las plantas anuales son las que presentan una mayor proporción de especies auto-compatibles; por su parte, los arbustos raramente presentan un rompimiento o una auto-compatibilidad completa y los árboles son auto-incompatibles (Figura 2). Sin embargo, cabe aclarar que el 78% de los estudios de sistemas de auto-incompatibilidad se han realizado en plantas herbáceas, por lo que se requiere de un número mayor de estudios en arbustos y árboles para identificar la asociación del rompimiento en el sistema de auto-incompatibilidad con una forma de vida.

Patrones por ambiente

El rompimiento en el sistema de auto-incompatibilidad es favorable al asegurar la producción de semillas en condiciones en que el intercambio de polen compatible puede ser raro (Levin, 1996). Se han registrado dos ejemplos de estas condiciones en Asteraceae. El primero está relacionado con tamaños poblacionales bajos y por ende una baja diversidad de alelos *S* en *Aster furcatus* (Reinartz y Les, 1994) y en *Eupatorium resinosum* (Byers, 1995). El segundo ejemplo está relacionado con condiciones ambientales que limitan la actividad de los polinizadores. Berry y Calvo (1989) reportan que las especies del género *Espeletia* que enfrenta ambientes más estresantes (temperaturas mínimas más bajas) presentan un rompimiento parcial en el sistema de auto-incompatibilidad.

Si consideramos que las zonas áridas pueden imponer restricciones a la actividad de los polinizadores debido a la incidencia de temperaturas extremas (superiores a los 30°C durante la época calurosa y menores a 0°C en la época fría) esperamos encontrar también una mayor frecuencia de especies que presenten un rompimiento parcial o sean auto-compatibles en comparación con las que se enfrentan a ambientes más mésicos. El análisis de las 226 especies de Asteraceae nos indica que en ambientes xéricos la incidencia de especies parcial o completamente auto-compatibles es mayor que en especies que habitan ambientes mésicos y que en las que se distribuyen tanto en ambientes mésicos y tropicales húmedos, y mésicos y templados (Figura 3).

En síntesis, la presencia de un sistema de auto-incompatibilidad es característica de la familia Asteraceae, pero la expresión de este sistema varía tanto con la forma de vida, como con el ambiente donde las especies habitan. El estudio de la expresión del sistema de auto-incompatibilidad es un campo abierto para explorar hipótesis ecológicas y evolutivas relacionadas con los sistemas reproductivos y la familia Asteraceae es un sistema idóneo para ponerlas a prueba, por su amplia distribución geográfica y la gran variación en la expresión del sistema de auto-incompatibilidad en sus diferentes especies.

Estructura genética en Asteraceae

La distribución de la variabilidad genética dentro y entre poblaciones –*i. e.* estructura genética- se ve afectada por el sistema reproductivo y el ambiente donde las especies habitan (Loveless y Hamrick, 1984; Nevo, 1986; Hamrick y Godt, 1990; Hamrick y Godt, 1996). Hamrick y Godt (1990) encontraron que en especies de plantas con polinización cruzada predominante, la variabilidad genética se encuentra dentro de las poblaciones y la diferenciación entre poblaciones es baja, sobre todo cuando el flujo génico es alto. Por otro lado, se espera que las especies que se enfrentan a ambientes inestables presenten una mayor diversidad genética (*i. e.*, heterocigosis) que aquellas que habitan en ambientes estables. Nevo (1986) hipotetiza, por lo tanto, que las especies de zonas áridas (ambientes xéricos) tienen una mayor diversidad genética que las especies de zonas templadas (ambientes mésicos).

Para identificar los patrones de variabilidad genética dentro de la familia Asteraceae relacionados con el ambiente, realizamos un análisis exploratorio de 65 especies en las que se ha descrito la estructura genética (índices H_S y G_{ST}; Nei, 1978) y de 243 especies en las que se han estimado diferentes índices de diversidad genética (*i. e.*, número de alelos por loci, heterocigosis esperada y proporción de loci polimórficos). Los datos se analizaron mediante una prueba de ANOVA para cada uno de los índices, en las que el factor independiente fue el ambiente en que las especies habitan (ambientes xéricos, mésicos, tropicales húmedos, mésicos y xéricos, y mésicos y tropicales húmedos). Las comparaciones múltiples de las medias se realizaron mediante una prueba de Tukey-Krammer para muestras desiguales, utilizando un nivel de confianza de 0.05.

El ambiente en que se dasarrollan las diferentes especies tiene una influencia marginalmente significativa sobre la distribución de la variabilidad genética en la familia ($F_{4,65}$ = 0.09475, P = 0.0516 para variabilidad genética dentro de poblaciones (H_S) y $F_{4,59}$ = 0.034821, P = 0.0054 para variabilidad genética entre poblaciones (G_{ST})). La variabilidad genética dentro de las poblaciones (H_S) y entre las poblaciones (G_{ST}) es en promedio baja (ca. 0.2) en todas las especies. Las pruebas de Tukey-Krammer separan sólo a la única especie que habita tanto en ambientes mésicos como tropicales húmedos (Figura 4). Esta especie (*Xanthium stramomium*) es auto-compatible, por lo que la divergencia entre las poblaciones (G_{ST} =0.93) y la baja variabilidad genética dentro de las poblaciones (H_S = 0.03) puede explicarse por el sistema reproductivo que le caracteriza.

En el resto de las especies analizadas, la divergencia entre poblaciones es baja ($G_{ST} \approx 0.2$). Este resultado se explica si consideramos que la polinización cruzada se ve favorecida por la presencia del sistema de auto-incompatibilidad y en las especies que son parcialmente auto-compatibles, lo que evita que las poblaciones se diferencíen. En este sentido la presencia del sistema de auto-incompatibilidad constituye una restricción filogenética que influye de manera importante en la variabilidad genética de las especies de la familia Asteraceae.

El ambiente en el que crecen las especies tiene un efecto significativo sobre los tres índices de diversidad genética (Tabla 1). Las comparaciones múltiples indican que las especies que crecen en ambientes mésicos tienen una menor proporción de loci polimórficos y una menor diversidad alélica (alelo/locus) que el resto de las especies que crecen en los otros ambientes (Figura 5). Las comparaciones múltiples indican que la heterocigosis esperada es baja en especies de ambientes mésicos, intermedia en especies de ambientes xéricos, en las de tropicales húmedos y en las de mésicos y xéricos, y alta en las que habitan tanto ambientes mésicos como tropicales húmedos.

La alta variabilidad genética encontrada en las especies de Asteraceae que habitan ambientes xéricos en comparación con aquellas que habitan en ambientes mésicos (Figura 4) apoyan la propuesta de que la diversidad genética es mayor en ambientes xéricos que en ambientes mésicos (Nevo, 1986) y sugieren que la diversidad genética de especies de zonas tropicales húmedas es tan variable como las de zonas áridas. En las zonas áridas la inestabilidad ambiental está muy relacionada con la impredecibilidad y variabilidad en la precipitación (Noy-Meir, 1990); mientras que en las zonas tropicales húmedas la dinámica de claros puede ser un factor importante que promueva una mayor diversidad genética (ver Alvarez-Buylla, 1990 y Eguiarte *et al.*, 1992).

Especie de estudio

Flourensia cernua D C. es una especie característica del Desierto Chihuahuense (Mac Mahon, 1988), dominante en varias comunidades vegetales (Rzedowski, 1981), y codominante en comunidades dominadas por gobernadora (*Larrea tridentata*), mesquite (*Prosopis* spp.) y tobosa (*Hilaria mutica;* Rzedowski, 1981; Montaña, 1988b). De las 13 especies del género *Flourensia* (Asteraceae: Heliantheae) presentes en Norteamérica, ésta es la más ampliamente distribuida (las otras 19 especies se distribuyen en Sudamérica, Dillon, 1984).

Flourensia cernua es un arbusto que llega a alcanzar hasta 2 m de altura, presenta tallos muy ramificados y se caracteriza por la presencia de una resina aromática en tallos y hojas (Dillon, 1984). Los adultos florecen a finales del otoño, fructifican en invierno y dispersan sus semillas al final del invierno. Tanto adultos como juveniles pierden las hojas durante el invierno y regeneran el follaje en verano, época en la que también se registra la germinación de las semillas (Mauchamp, 1992).

Las flores hermafroditas de *F. cernua* son protándricas, no presentan néctar y se agregan en cabezuelas solitarias que se incurvan hacia el suelo, lo que facilita la liberación del polen en el aire (que ocurre entre las 11 y 14 horas solares, Mauchamp, 1992). Los aquenios pequeños (4 a 6.5 mm de largo) son vellosos y presentan un pappus con dos ganchos (Dillon, 1984). Cuando el fruto, ya sea inmaduro o maduro, es consumido por ovejas, cabras, y/o ganado vacuno, causa intoxicación y hasta la muerte por envenenamiento debido a los terpenoides que se producen en él (Dollahite y Allen, 1975).

La especie sufre de una baja herbivoría tanto por vertebrados, como por invertebrados. El sabor picante y amargo del follaje del arbusto lo hacen desagradable al gusto del ganado y de la fauna silvestre, por lo que raramente es consumida por el ganado vacuno, lo cual ocurre sólo cuando especies más palatables no están disponibles (Schmutz *et al.*, 1968). A pesar de ello los individuos juveniles pueden perder todo su follaje al ser consumido por liebres (Mauchamp, 1992; Gibbens *et al.*, 1996). A lo largo de su área de distribución geográfica 88 especies de insectos fitófagos de 35 familias se asocian con esta especie (Richerson y Boldt, 1995). Estos autores encuentran que sólo *Goinatrum planum* Bruner (Orthoptera: Acridideae) y *Buccalatrix flourensiae* Braun (Lepidoptera: Lyonetiidae) son fitófagos específicos, 39 son polífagas, 11 olífagas, mientras que de las 36 especies restantes se desconoce su especificidad. La mayoría de las especies de insectos se alimentan de las hojas y tallos (83 de las 88) y una minoría lo hace de flores y frutos (9 de las 88); otras especies de artrópodos como arañas, abejas, hormigas, avispas y otros depredadores se encuentran comúnmente asociadas a *F. cernua* (Richerson y Boldt, 1995).

Algunos trabajos realizados a principios de la década de los 80's enfatizaron el papel de *F. cernua* en la intercepción del agua de precipitación, considerando que al menos 6% del total del agua de precipitación se interceptaba por el follaje y se perdía por evapotranspiración (Tromble, 1983, 1987). Sin embargo, estudios recientes demuestran que la arquitectura de la planta promueve un flujo caulinar y permite la humidificación de los suelos, factor importante en la generación de islas de recursos (Mauchamp y Janeu, 1993; Martínez-Meza y Whitford, 1996). Esta redistribución del agua puede tener también un efecto positivo en el reclutamiento de *F. cernua*, puesto que las semillas tienden a dispersarse cerca de la planta madre (Mauchamp *et al.*, 1993) y sus posibilidades de germinar dependen de la humedad del sustrato (Valencia-Díaz y Montaña, 2003).

Dos trabajos relacionados con la germinación de esta especie indican que sus semillas germinan en condiciones de alta humedad, sobreviven a amplio intervalo de temperaturas (de 5° a 50°C) y las plántulas pueden establecerse en zonas abiertas, donde la radiación solar es alta (Mauchamp *et al.*, 1993; Valencia-Díaz y Montaña, 2003). Las semillas de *F. cernua* son quiescentes y tienen una longevidad menor a dos años, lo que podría favorecer la creación de un banco de semillas temporal y permitir el establecimiento de plántulas cuando las condiciones sean favorables (Valencia-Díaz y Montaña, 2003).

Flourensia cernua puede establecerse tanto en suelos jóvenes como maduros, principalmente en aquellos con un horizonte superficial de grava, aunque también se le encuentra en suelos someros que presentan un horizonte petrocálcico de hasta 90 cm de profundidad, al anclar sus raíces en las grietas que se forman en éste (Gile *et al.*, 1998).

A *F. cernua* se le conoce en México como *hojase, hojasen* y *ojasen* y en Estados Unidos de Norteamérica como *tarbush, blackbush* y *varnish bush*. En el norte de México las hojas y cabezuelas de los individuos de esta especie se han usado para hacer una cocción para tratar la indigestión (Dillon, 1984; Ferrer, 1996 y citas ahí). A la especie se le considera una invasora de tierras sobre-pastoreadas en el suroeste de Estados Unidos y se piensa que disminuye la productividad de matorrales y pastizales de valor forrajero para el ganado (Gibbens *et al.*, 1993; Gibbens *et al.*, 1996a, 1996b; Gile *et al.*, 1998). Como otros arbustos que han invadido comunidades dominadas por pastizales, *F. cernua* está sujeta a planes de erradicación para evitar la pérdida de agua por evapotranspiración causada por estos arbustos (Texas Soil and Water Conservation State Board 2002). Sin embargo, la información sobre el balance energético e hídrico de las comunidades donde *F. cernua* es la especie dominante revelan que no existen diferencias en la evapotranspiración entre estas comunidades vegetales y aquellas en que dominan las herbáceas perennes (Gibbens *et al.*, 1996b).

Sitio de estudio

Este trabajo se realizó en la Reserva de la Biósfera de Mapimí, Durango, México. Esta reserva se encuentra en el sudoeste del Desierto Chihuahuense (26° 40' N y 103° 40' W). En ella se presentan 14 formaciones vegetales caracterizadas por la dominancia de alguna forma de vida (*e. g.* leñosas altas, leñosas bajas y herbáceas perennes) y 74 asociaciones, dependiendo de la dominancia de una especie u otras (Montaña, 1988a). Los trabajos del presente estudio se realizaron en dos de estas formaciones que se distribuyen de manera contigua en las bajadas inferiores del Cerro San Ignacio y que se describen en los siguientes párrafos. Los suelos de ambos formaciones son Xerosoles calcícos y lúvicos desarrollados en depósitos aluviales que se caracterizan por la acumulación de material grueso y por el transporte de sedimentos finos y sales, y por ser los suelos más desarrollados y profundos de las planicies de la Reserva de la Biósfera de Mapimí (Breimer, 1988).

La primera formación vegetal se caracteriza por la dominancia de la gramínea *Hilaria mutica* en el estrato de herbáceas perennes, con la presencia de un importante estrato de arbustos dominado por *Prosopis glandulosa* y *Flourensia cernua* (formaciones 5.1 en Montaña, 1988b).

Estas formaciones se ubican en zonas con pendientes menores a 1.5% y se conocen como "mogotes" o "arcos de vegetación" y se presentan como bandas de vegetación distribuidas de forma perpendicular a la pendiente (Montaña *et al.*, 2001); lo que en este estudio se denominó como matorrales densos. La dinámica de la vegetación de los matorrales densos ha sido ampliamente estudiada y en términos generales puede ser descrita como un mosaico de dos fases dependiente del agua de escurrimiento: una de vegetación densa y otra de suelo casi desnudo (Montaña, 1992; Montaña *et al.*, 2001). La fase de vegetación densa está constituida por tres zonas: la frontal ubicada pendiente arriba, la central y la terminal ubicada pendiente abajo; en la zona frontal se dan procesos de colonización, mientras que en la zona terminal, la vegetación muere, lo que origina un proceso de sucesión y promueve el movimiento de las bandas de vegetación hacia arriba(Montaña, 1992; Montaña *et al.*, 2001).

La segunda formación vegetal se caracteriza por la dominancia de *Larrea tridentata* y ocasionalmente por la codominancia de *Prosopis glandulosa* var. *torreyana* y *Cordia parviflora,* un segundo estrato pobre en el que aparece *Opuntia rastrera* y ocasionalmente *O. leptocaulis* y *O. microdasys,* y un tercer estrato muy variable con la presencia constante de *Jatropha dioica* (formaciones 6.1 y 6.3 en Montaña, 1988b). Estas formaciones se ubican en pendientes de más de 1.5%, se conocen como "nopaleras" y corresponden a lo que en este trabajo se denomnó como matorral difuso. En estas zonas *F. cernua* se encuentra distribuida a lo largo de arroyos temporales.

Características climáticas

El clima de la región es árido tropical con lluvias de verano, con una precipitación anual promedio de 264 mm; el 76% de la precipitación se presenta entre junio y septiembre y la frecuencia de eventos de precipitación mayores a 10 mm es de aproximadamente 18% (Cornet, 1988). La temperatura media anual es de 20.8°C. Sin embargo, las temperaturas presentan una gran variación estacional: en el invierno la temperatura mínima promedio es de 3.9°C y se registran heladas en un promedio de 13 días al año. En el verano la temperatura máxima promedio es de 36.1°C y se registran temperaturas mayores a 30°C en un promedio de 14 días al año (Cornet, 1988).

El análisis de los datos de clima de la década de 1991-2000 indica que el promedio de la precipitación acumulada anual (218.85 mm) es menor que el promedio reportado por Cornet (1988), lo que sugiere que un período de sequía largo ha afectado la zona. En la década pasada, sólo dos años (1991 = 344.9 mm y 2000 = 292.6) tuvieron una precipitación mayor al promedio reportado por Cornet (1988, Figura 6). La proporción de eventos que pueden producir un escurrimiento superficial importante de agua (precipitación pluvial > 10 mm) fue en promedio del 20% para la década, sin embargo existe una gran variación interanual para este parámetro (rango, 7% - 33%; valores de 1997 y 1996 respectivamente, Figura 6).

Durante la época reproductiva de *F. cernua* (de noviembre a febrero) la variación climática fue también considerable en la década de 1991 a 2000. La precipitación total acumulada en esta época fue en promedio de 29 mm, con la presencia de un invierno inusualmente húmedo de 1991 a 1992 (150.8 mm). El resto de los inviernos fue extremadamente seco (16.9 mm en promedio), de hecho, sólo el 4% de los eventos de precipitación ocurrieron en esta época en el período de 1992 a 2000. Los inviernos fueron ligeramente más cálidos que los de las décadas pasadas (4.8°C vs. 3.9°C, *cf.* Cornet, 1988). Sin embargo, en el período de 1997 a 1998 el invierno fue más frío (temperatura mínima promedio de 3.14 ° C) y en éste se registró el día más frío desde 1983 (-8.5°C vs. –7.5°C en 1983, *cf.* Cornet, 1988; Figura 7).

Planteamiento del problema

La reproducción de especies que no presentan reproducción vegetativa depende completamente de la producción de semillas. En este sentido es importante investigar qué factores pueden limitar la reproducción sexual de la especie en tres fases fenológicas:1) polinización, 2) maduración de frutos y semillas, y 3) germinación de semillas.

La densidad es un atributo poblacional que puede afectar la adecuación de los individuos (Harper, 1977). En especies anemófilas una mayor densidad poblacional incrementa la disponibilidad de polen (Allison, 1990) y las tasas de polinización cruzada (Farris y Miton, 1984). Este incremento en la disponibilidad de polen, y las tasas de polinización cruzada se traduce en una mayor adecuación femenina en plantas que dependen de polen exógeno para producir semillas (Arista y Talavera, 1996).

En la Reserva de la Biósfera de Mapimí *F. cernua* se distribuye en dos tipos de matorral que se presentan de manera contigua y que contrastan en su densidad poblacional (matorrales densos y difusos). Este arbusto no presenta reproducción vegetativa, tiene una baja producción de semillas viables y su síndrome de polinización es anemófilo. De esta forma constituye un sistema ideal para explorar hipótesis sobre los efectos de la densidad poblacional en especies anemófilas.

Objetivo general

Comparar las características de la biología reproductiva y de la genética de poblaciones en dos matorrales con densidad poblacional contrastante de *F. cernua.*

Objetivos particulares

Estimar y comparar los componentes de adecuación femenina asociados a la fase de polinización, maduración de frutos y germinación de semillas de los individuos que habitan en el matorral difuso y denso.

Caracterizar la presencia de sistemas de auto-incompatibilidad y los efectos de la polinización cruzada y auto-polinización en la producción de semillas de *F. cernua* en las poblaciones de matorral denso y difuso.

Estimar las tasas de polinización cruzada y estimadores de diversidad y estructura genética de las poblaciones de matorral difuso y denso.

Hipótesis

Dadas las diferencias en densidad poblacional de los matorrales de *F. cernua* se espera: 1) una mayor adecuación femenina en los matorrales densos, 2) tasas de polinización mayores en los matorrales densos y 3) una diferenciación genética entre los matorrales densos y difusos como consecuencia de las diferencias en las tasas de polinización.

Tabla 1. Resultados de los análisis de varianza para tres índices de variabilidad genética (proporción de loci polimórficos, número de alelos por locus y heterocigosis esperada) para los datos de diferentes estudios de genética de poblaciones en la familia Asteraceae agrupados según el ambiente (Zonas áridas (Xérico), Zonas áridas y templadas (MX), zonas templadas (Mésico), zonas templadas y tropicales húmedas (MT) y zonas tropicales húmedas (Trópico) en donde crecen las especies.

	Fuente de variación	g. l.	CM efecto	CM error	F	p
Proporción de loci polimórficos	Ambiente	4, 243	0.171	0.035	4.913	0.0008
Alelos / locus	Ambiente	4, 230	1.754	0.215	8.155	0.0000
Heterocigosis esperada	Ambiente	4,65	0.024	0.009	2.491	0.0516

Figura 1. Esquema que representa la reacción de auto-incompatibilidad esporofítica, con cuatro alelos. El genotipo materno para todos los casos es S_1S_2 y el genotipo del padre se encuentra en el círculo grande, los círculos pequeños representan el grano de polen en los que se especifica su genotipo haploide. Sobre los estigmas se presentan las reacciones incompatibles, como aquellas en las que no hay germinación y penetración del tubo polínico y las compatibles como aquellas en las que el polen germina y penetra la superficie estigmática.

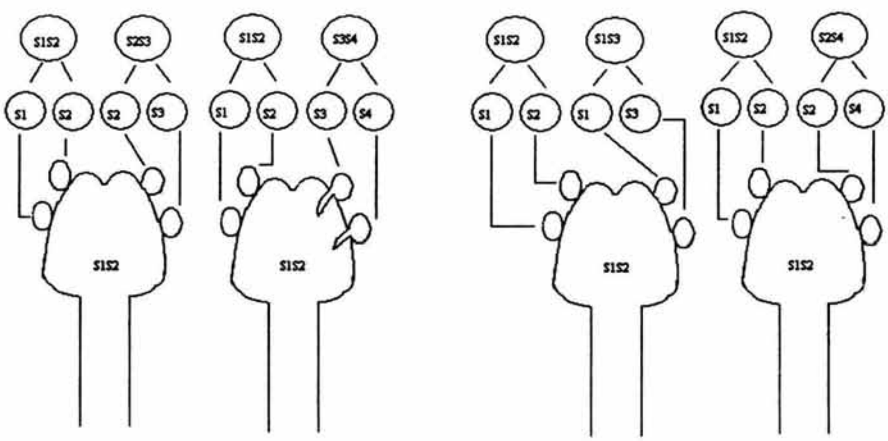


Figura 2. Distribución de 226 especies de Asteraceae agrupadas por forma de vida (las herbáceas se dividieron en anuales, bianuales y perennes, y aquellas que pueden ser tanto anuales como perennes -anual-perenne en la figura-) y por categoría del sistema de auto-incompatibilidad (incompatible, parcialmente incompatible –presentan un rompimiento, pero la producción de semillas de auto-polinización es significativamente menor a la de polinización cruzada- y auto-compatible, la producción de semillas de auto-polinización y polinización cruzada son iguales).

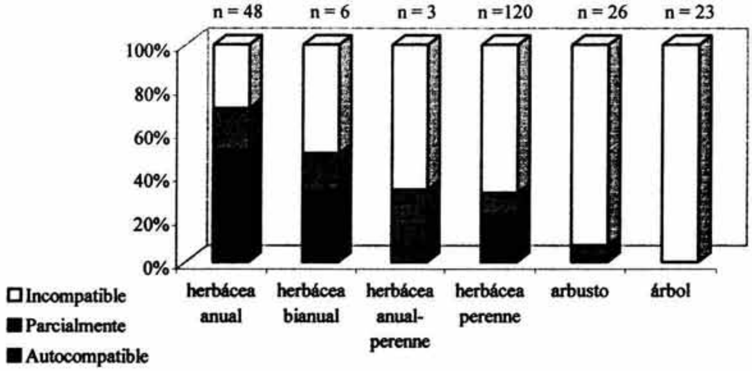


Figura 3. Distribución de 226 especies de Asteraceae agrupadas por ambiente y categorías de sistemas de auto-incompatibilidad: incompatible, parcialmente incompatible (presentan un rompimiento, pero la producción de semillas de auto-polinización es significativamente menor a la de polinización cruzada) y auto-compatible (la producción de semillas de auto-polinización y polinización cruzada son iguales.

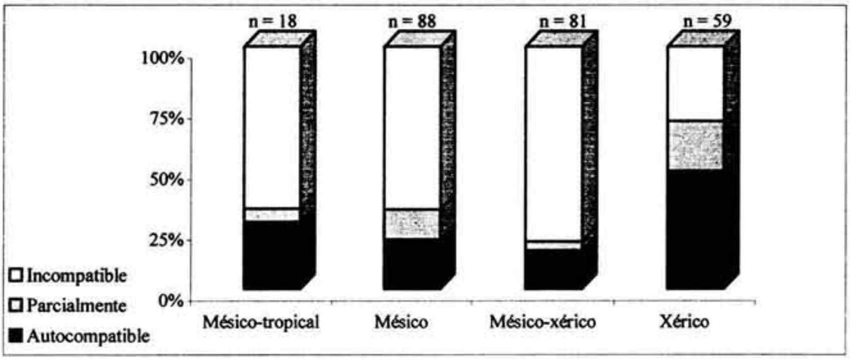


Figura 4. Variación genética dentro de poblaciones (H_S) y entre poblaciones (G_{ST}) en 60 especies de Asteraceae agrupadas por ambientes. Los ambientes son: Zonas áridas (Xérico), Zonas áridas y templadas (MX), zonas templadas (Mésico), zonas templadas y tropicales húmedas (MT) y zonas tropicales húmedas (Trópico)

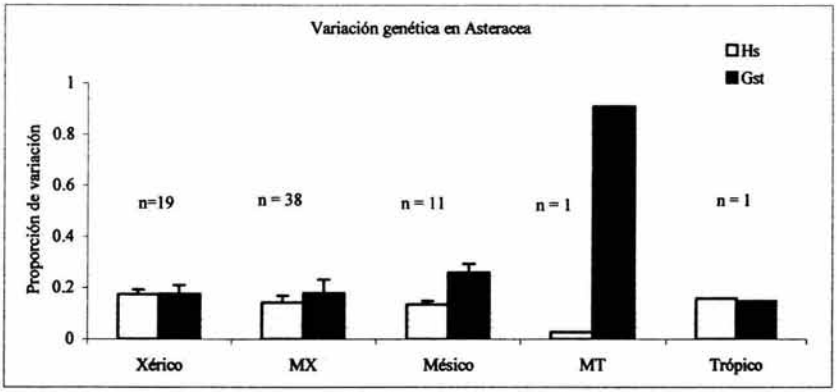


Figura 5. Medias (+ un error estándar) de los índices de diversidad genética: proporción de loci polimórficos (Polimorfismo), diversidad alélica (número de alelos por locus) y heterocigosis esperada (He) para 223 especies de Asteraceae agrupadas por ambientes. Zonas áridas (Xérico), Zonas áridas y templadas (MX), zonas templadas (Mésico), zonas templadas y tropicales húmedas (MT) y zonas tropicales húmedas (Trópico). Los asteriscos indican diferencias significativas entre ambientes para cada índice de diversidad genética (prueba de Tukey-Krammer $P < 0.05$)

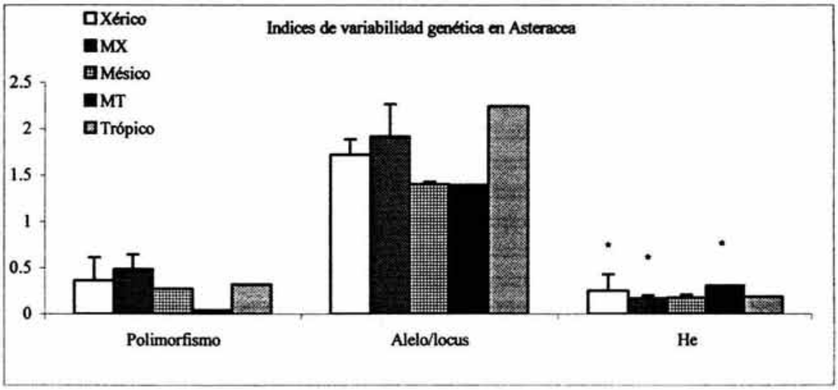


Figura 6. Precipitación pluvial total durante la época reproductiva (ER) de *F. cernua* (noviembre-febrero), precipitación total anual y porcentaje de eventos que pueden producir escurrimientos para los años 1991-2000, en la zona de estudio. Datos de la estación meteorológica del Laboratorio del Desierto.

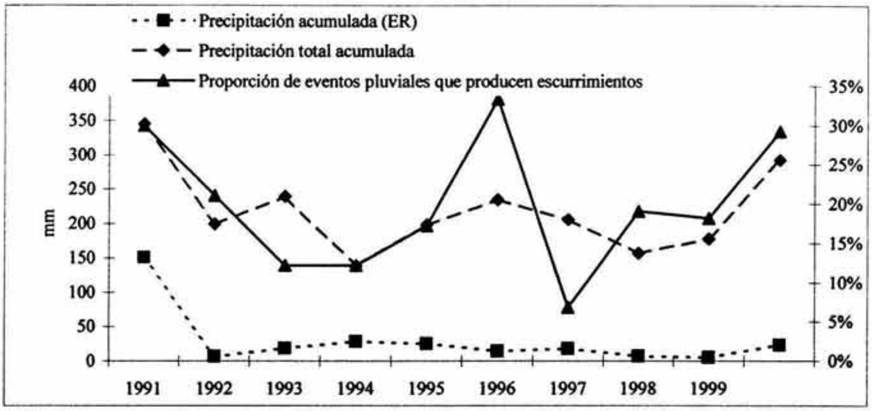
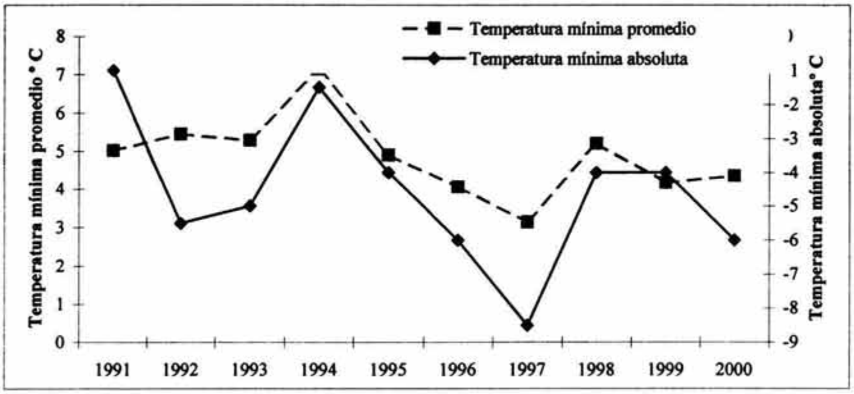


Figura 7. Temperatura mínima promedio y temperatura mínima absoluta para la época reproductiva de *F. cernua* (noviembre a febrero) en la zona de estudio. Datos de la estación meteorológica del Laboratorio del Desierto.



Effects of plant spacing and flowering synchrony in the reproductive efficiency of the Chihuahuan Desert shrub *Flourensia cernua* (Asteraceae).

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Reproductive potential of *F. cernua*

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Abstract

- **Background and aims**: Reproductive potential could be limited by low pollen and resources availability, selective abortion of fruits and seeds, predispersal predation, self-incompatibility systems and inbreeding depression. Another factor that could limit reproductive potential in outcrossing plants is the limited availability of potential mates, which in turn is affected by flowering synchrony and spacing among reproductive neighbors. In this paper the relative contribution of six female fitness components to the reproductive potential and their variation through of time, scrub type, flowering synchrony and spacing among nearest reproductive neighbors was addressed in the desert shrub *Flourensia cernua*.
- **Methods**: At each one of five dense-scrub sub-plots and five diffuse-scrub sub-plots, five randomly selected stems in each of 10 randomly selected plants were monitored to assess fitness components during two years. For each plant a synchrony index and the mean distance to the four nearest reproductive neighbors were also measured to assess its effect on female fitness.
- **Key Results:** The production of viable seeds was very low and this limitation of the reproductive potential was associated to four main processes: a) predation by beetles (ca. 25% of initial flowers), b) pollination failures (ca. 25% of non predated flowers), c) abortion (ca. 90% of initiated fruits) and d) low germinability rates (<10% seeds germinated). Higher reproductive succes was found when plants grew more clumped and bloomed synchronously.
- **Conclusions:** Reproductive potential in *F. cernua* is limited by factors such as low seed viability, abortion, predispersal seed predation and mate availability (determined by plant

spacing and flowering synchrony). We speculate that natural selection is favoring phenological traits that promote flowering synchrony of individuals.

Key words: Breeding structure, *Flourensia cernua*, flowering synchrony, fruit set limitations, population density, seed set limitations.

INTRODUCTION

Among the best-documented factors that may limit reproductive potential in plants are low pollen and resource availability (Lovett-Doust and Lovett-Doust, 1992; Burd, 1994; 1995; 1998; Larson and Barret, 2000), selective abortion of fruits and seeds (Stephenson, 1980, 1981), predispersal predation (Ayre and Whelan, 1989; Lovett-Doust and Lovett-Doust, 1992), self-incompatibility systems (Sutherland and Delph, 1984, Sutherland, 1986) and inbreeding depression (Charlesworth and Charlesworth, 1987).

Another factor that could limit the production of viable seeds in self-incompatible plants or in those that benefit from high rates of cross-pollination is the limited availability of potential mates (Bawa, 1983). In such species, mate availability is affected by at least two factors: flowering synchrony (i. e., the degree of temporal overlap in flowering of the individuals within a population, Augspurger, 1981; Ratchke and Lacey, 1985), and spacing among reproductive neighbors. These two factors are components of the population's breeding structure and can affect plant fitness (Levin, 1988). It has been reported that when flowering synchrony increases, seed set increases in species such as: *Claytonia virginica* (Schemske, 1977), *Manfreda brachystachya* (Eguiarte and Búrquez, 1987), and *Erythroxylum havanense* (Domínguez and Dirzo, 1995); in the latter case this was true only in one out of the two years of study. Seed set increases and seed predation decreases as population density increases in *Hybanthus prunifolius* (Augspurger, 1981); and Ollerton and Lack (1998) found that in *Lotus corniculatus* seed predation decreases as flowering synchrony increases only in one out of the three years during which they studied the population of this species.

The spacing among reproductive neighbors can also have an important influence on female fitness in anemophilous species. In these species, close spacing among reproductive neighbors is often associated with an increase in fruit set and/or seed set (i.e. Allison, 1990; Smith *et al.*, 1988;

Morgante *et al.*, 1991; Arista and Talavera, 1996), with higher production of viable seeds (Morgante *et al.,* 1991; Arista and Talavera, 1996), with lower seed predation (i.e., Janzen, 1971; 1976) and/or lower fruit abortion (Allison, 1990).

In arid and semiarid areas the recruitment of perennial plants is a sporadic event despite the fact that many species produce abundant seed crops (Shreve, 1929; Jordan and Nobel, 1979; Eguiarte and Búrquez, 1987; Nobel, 1988; Cody, 1993; Mandujano *et al.,* 1995). In some cases, despite a high potential fecundity (number of viable seeds per unit area) recruitment is low due to environmental factors such as variable and unpredictable climatic events that affect seed germination and seedling survival (Shreve, 1917; Beatley, 1974; Ackerman, 1979; Nobel, 1985, 1988; Bowers, 1994; Bowers *et al.,* 1995). In other cases, potential fecundity is limited by low reproductive efficiency (viable seeds / initial flowers) which can be caused by a variety of factors as described above. Some studies found high seed abortion rates, as in *Larrea tridentata* (Barbour, 1968), or low production of viable seeds, as in *Maireana sedifolia* (Crisp, 1978) and *Atriplex vesicaria* (Hunt, 2001), but there are few studies that quantitatively document the female fitness components in semiarid plants (e. g., Eguiarte and Búrquez, 1987; Mandujano *et al.,* 1995; Hunt, 2001) and none of them assessed the influence of flowering synchrony and spacing among reproductive plants on fitness components.

Flourensia cernua (Asteraceae: Heliantheae) is shrub characteristic of the Chihuahuan Desert, and is dominant in several plant communities (Rzedowski, 1978; Dillon, 1984). However, previous work indicated that the recruitment of new seedlings in its population is very low (C. Montaña pers. obs.) and that seeds have low viability (germinability) compared to other arid land shrubs of the Asteraceae (Valencia-Díaz and Montaña, 2003). In this paper, we study the spatial and temporal variability of fitness components and the dependence of them on the flowering synchrony and the spacing between reproductive individuals. Using repeated measures ANOVAs we examine the spatial and temporal variation of six female fitness components that affect seed production in two scrub types which exhibit contrasting plant density during two reproductive seasons. Three of the female fitness components we studied pertain to the phenological phase of flower pollination, one of them to stages in fruit ripening and two to seed germination. Simultaneously, using the same plants from both scrub types, we examined whether individuals are reproduction limited due to a paucity in compatible mating partners by analyzing the multiple regression of female fitness on spacing among reproductive neighbors and on the proportion of synchronously flowering individuals. Specifically, the relative contribution of the six fitness components to the reproductive potential and the variation of those fitness components as a function of time, scrub type, flowering synchrony and spacing among nearest reproductive neighbors was addressed in this paper.

MATERIAL AND METHODS

Study site and Species

Fieldwork was carried out in the Mapimí Biosphere Reserve (Durango, México). This Reserve is located in the southwest of the Chihuahuan Desert, 26° 40' N and 103° 40' W; 1,100 m a.s.l., with an average annual precipitation 264 mm, 72% of which falls between June and September (Cornet, 1988). The annual precipitation for the years of study was 205.9 mm and 138 mm in 1997 and 1998, respectively.

Flourensia cernua is distributed in two contiguous scrub types both of which are found in *bajadas* (gentle sloping terrain connecting the base of hills with the bottom of endorreic basins): dense scrubs (vegetation arcs or "brousse tigrée" described by Montaña *et al.,* 1990), and diffuse scrubs. The dense scrubs do not differ from diffuse scrubs in general climatic or edaphic characteristics, except for a slightly higher clay content in the soil and higher water availability due to runoff rain-water redistribution (Cornet et al., 1992, Montaña, 1992, Galle et al., 2001). The diffuse scrubs establish on slopes > 3% and most plants of *F. cernua* are distributed along temporary streams. Density of reproductive adults is 81 individuals/ha ± 0.5 SE (measured in five ≈ 1.5-ha permanent plots used for long term demographic studies). The dominant species (according to cover values) are *Larrea tridentata*, *Opuntia rastrera* and *Jatropha dioica*. The dense scrubs ocurr on slopes <1.5%; where the density of reproductive adults is 878 individuals/ha ± 35 SE (measured in five ≈ 0.4-ha permanent plots used for long term demographic studies). The dominant species (according to cover values) at the sites are *Prosopis glandulosa* var. *torreyana*, *Hilaria mutica* and *F. cernua*. All reported permanent plots were randomly selected in each scrub type based on the vegetation map of the Mapimí Biosphere Reserve (Montaña, 1988) over an area of 20 km^2 (7 km^2 of dense scrubs and 13 km^2 of diffuse scrubs).

Flourencia cernua, does not propagate vegetatively and has hermaphroditic flowers that are wind pollinated (Dillon, 1984; Mauchamp, 1992). Microscopic observation of pollen tube development after controlled pollination lead Ferrer et al. (chapter two) to conclude that a self-incompatibility system operates in the species. They also found evidence of pseudo self-fertilization (sensu Levin, 1996), that is, some individuals can produce fruits after self-fertilization, but offspring derived from cross-pollination have a larger fitness than those derived from self-pollination. As in many species of Asteraceae, the flowers are protandrous and each flower produces only one ovule, and each inflorescence bar approximately 14 to 25 flowers. Flowers open at the end of the autumn (from October to December, Dillon 1984), anther dehiscence occurs during the first two days of flowering, while on the second day the style begins to elongate, and the stigma becomes receptive from the third to the fifth day of flowering (determined through microscopic observations by M. Ferrer, unpub. res.). Although nine insect species have been reported feed upon flowers and fruits over the geographic range of *F. cernua* (Richerson and Bolt, 1985), we found only three insect species all of which feed on flowers: *Smicronyx profusus* Casey, *S. spretus* Dietz (Coleoptera:Curculionidae) and *Euraestoides acutangulus* (Thomson) (Diptera: Tephritidae). Occasional outbreaks of a fourth species, *Camplyacantha olivaceae vivax* (Scudder) (Orthoptera: Acrididae), which primarily feeds pimarily on vegetative buds but can also prey on reproductive buds have also been observed. Within the study site, the larvae of *S. profusus* causes the largest damage to fruits. Females of *S. profusus* lay their eggs on floral buds of *F. cernua*, approximately one month before anthesis. The larvae then feed on the ovaries and embryos of the whole floral head in which they develop and then fall to the ground to continue development in the soil. The other curculionid and the tephritid only feed on the ovary in which the egg was ovoposited and therefore they do not destroy the whole floral head (Mauchamp, 1992; M. Ferrer and C. Montaña, pers. obs.).

Seed ripening and dispersal of *F. cernua* occur in winter (January to March, Dillon, 1984). Seeds may germinate immediately after dispersal. Viability is low (less than 10% of filled seeds) and could not be improved with scarification, stratification or gibberelic acid treatments (Valencia Díaz and Montaña 2003).

Spatial and temporal variation in female fitness components

Measurements were made in five ≈ 1.5-ha permanent plots used for long term demographic studies in diffuse scrub and five ≈ 0.4-ha permanent plots used for long term demographic studies in dense scrub. Female fitness components were measured in one study sub-plot located arround the centre of each of these permanent plots. In each sub-plot, ten randomly chosen plants were tagged and observed during the reproductive season (October to February) in both 1997 and 1998. In both years five different flowering stems (representing between 30% and 50% of the total number of flowering stems, each stem sustented arround 30 flower heads) were randomly chosen on each tagged plant. At the end of the flowering phenological phase, the five stems of each plant were individually bagged with a fine mesh. After bagging, during the fruit ripening phenological phase, many of the aborted and ripe fruits fell from the flower head and were recovered in the bags. After the end of the fruit ripening phenological phase, all reproductive structures contained in each bags were collected and the numbers of the following reproductive structures were recorded: 1) flowers (total number of flowers, *i. e.* the sum of 2, 3, 4 and 5), 2) non-pollinated flowers (all flowers that withered without the development of an embryo), 3) insect damaged fruits, 4) aborted fruits (achenes with an undeveloped embryo occupying less than 75% of the achene volume and whose walls collapsed and broke when applying light pressure with forceps), 5) fruit with seeds (achenes with a fully developed embryo occupying almost all of the achene volume). As the identity of the particular flower head that produced the detached fruits collected in the bags could not be retrieved, it was not possible to express the numbers of the reproductive structures on a per flower head basis and they were expressed on a per stem basis instead. Retrieved seeds were germinated on humid cotton in environmental chambers under the conditions recommended by Mauchamp (1992) and Valencia-Díaz and Montaña (2003), i.e., with a photoperiod of 14 hours light at 26°C and 10 hours darkness at 16°C and 60% environmental humidity. The number of germinated seeds was recorded after 20 days (when most viable seeds were germinate according to Mauchamp 1992 and Valencia-Díaz and Montaña 2003).

These data were used to compute the values of six female fitness components. Three of them pertain to the phenological phase of flower pollination: 1) number of flowers, 2) predation: ratio of flowers damaged by predators to initial flowers, 3) fruit set: ratio of fruits to initial flowers minus flowers damaged by predators. Another one of them pertain to the fruit ripening phenological phase: 4) seed set: ratio of seeds to fruits. And the last two to seed germination: 5) reproductive efficiency: ratio of germinated seeds to initial flowers, 6) viable seed production per plant (reproductive efficiency*number of flowers). Reproductive efficiency allows easy comparison between species, because it is expressed as a proportion. However, viable seed production is also a useful measure because it takes into account the between plant variation in number of flowers.

We assessed the variation of each one of the components of female fitness (except the first and the last one) by using repeated-measures ANOVAs (implemented through a MANOVAR procedure, see Potvin, *et al.*, 1990, also called MANOVA procedure Von Ende, 1993). The main effects were year (within subject effect) and scrub type and both of them were treated as fixed effects factors. Sub-plots were nested withi scrub type and individuals were nested within the sub-plots and scrub type. Sub-plot and individual were considered as random effects factors.

To asses variation in the production of flowers and of viable seeds, the response variable was expressed on a per plant basis by multiplying the number of stems bearing floral heads by the average of the number of flowers and the reproductive efficiency of the five selected stems. Thus, beside the fixed-effects factors scrub types and year (within-subject effect), this model had only sub-plot nested in scrub type as random effect factor.

To make the residuals normally distributed, an angular transformation (arcsine $\sqrt{p}$, where p is a ratio value) was applied to the variables expressed as ratios values (Sokal and Rohlf, 1995). Multiple comparisons were made using the Tukey- Krammer test (Sokal and Rohlf, 1995). All calculations were carried out with the statistical program STATISTICA (v. 4.3, Statsoft. Co., 1993).

Breeding structure and reproductive potential.

Two components of the population breeding structure: spacing among reproductive neighbors and flowering synchrony, were estimated for each of the 10 focal individuals per sub-plot per scrub type (i.e., 50 individuals in dense and 50 individuals in diffuse scrubs). The spacing among reproductive neighbors was estimated as the average distance from each focal individual to the four nearest reproductive adults. The flowering synchrony was calculated with the index proposed by Augspurger (1983, modified of Primack, 1980). The synchrony index X for an individual (i) is:

$$X_i = (1/n-1)(1/f_i)\sum_{i=1}^{n} e_{ij\neq i}$$

where: $i = 1, 2, \ldots\ldots 10$ are the 10 selected individuals of each sub-plot; $j = 1, 2, \ldots\ldots\ldots n$ are the n plants of each plot of demography survey (n varied between 30 and 70 plants); e_{ij} is the number of days in which the selected individual i and its j neighbor overlaped in their flowering; f_i is the number of days in which individual i was flowering and n is the number of individuals in the sample.

The synchrony index ranges from zero (when an individual's blooming is asynchronous with the rest of the individuals in the population) to 1 (total synchrony). This index supposes that the number of active flowers is constant through time. To control for this, the days at the beginning and at the end of the blooming period of each plant, when less than 5% of the flowers were open, were excluded from the analysis (cf. Augspurger, 1983; Gómez, 1993; Ollerton and Lack, 1998). The presence of active flowers was monitored every other day.

The between scrub-type and between year variability of the synchrony index was analyzed on a per plant basis with the same statistical model used for the number of flowers. The between scrub-type variability of the spacing among reproductive neighbors was analyzed with a nested ANOVA (individuals nested in sub-plots, sub-plots nested in scrub types). Individual and sub-plot were considered as random-effect factors while scrub type was a fixed-effect factor. Obviously, the between year variability of spacing among reproductive neighbors could not be analyzed because reproductive neighbors were the same in both years.

Additionally to the comparison of mean spacing among reproductive neighbors assessed through the ANOVA *F*-test, the frequency distribution of plant spacing in both scrub types was compared through a Chi-square test. Frequency intervals were delimited each 10 m.

The effects of spacing between neighbors and floral synchrony on each fitness component were assessed through multiple regressions using a forward stepwise procedure. In each step a variable was included only if its inclusion increased by 1 or more the *F*-value. A separate regression was made with the data comprised in each treatment (combination of scrub type and year) except when results of the repeated measures ANOVAs of the fitness component indicated no differences between years or between scrub types, in which cases data of non different treatments were pooled. To avoid the use of pseudoreplicates in the regressions, the fitness components were calculated on a per plant basis by averaging the value of the five tagged stems (i.e. $n = 50$ for each combination of scrub type and year). Values of the spacing among reproductive neighbors were transformed to a 0 to 1 scale dividing each of them by the maximum recorded value. In this way spacing and synchrony values had the same range of variation making the comparison of partial regression coefficients and slope values more meaningful. By a similar reasoning the same transformation was applied to the numbers of flowers and numbers of viable seeds to facilitate the comparison between the regression parameter of all fitness components. The analyses of these data were carried out with the program STATISTICA (v 4.3 Statsoft Co., 1993).

RESULTS

Spatial and temporal variation in reproductive potential

The production of viable seeds was very low although plants had a large floral display (5,963 flowers ± 278 SE after pooling all the data, i.e. $n = 200$ plants) which did not vary among years or scrub types (Table 1a).

The significant interaction between scrub type and year is related to the fact that flower predation in 1998 was similar in both scrub types (11.47% ± 2.24 SE, $n = 250$ stems, Tukey test $P > 0.05$), but in 1997 flower predation was almost twice as high in the diffuse than in the dense scrubs (39.94% ± 3.45 SE vs. 19.79% ± 3.18 SE respectively, in each case $n = 250$ stems, Tukey test $P < 0.001$, Table 1b, Fig. 1). Fruit set differed among years (75.55% ± 1.4 SE for 1997 and 71.40% ± 1.3 SE for 1998, Table 1b, in each case $n = 500$ stems, F- test $P = 0.04$) but did not differ between scrub types (F- test $P = 0.25$, Table 1b).

On the other hand, seed set did not differ between years nor between scrub types (9.55% ± 1.97 SE, $n = 1{,}000$ stems, F- test $P = 0.34$ and 0.92 respectively, Table 1b). Variation in reproductive efficiency was influenced only by year (F- test $P = 0.003$, Table 1b). None of the 1997 seeds from either scrub type germinated and, consequently, reproductive efficiency was zero. In 1998 the reproductive efficiency did not differ among scrub types (0.48% ± 0.06 SE, $n = 500$ stems, F- test $P = 0.36$, Table 1b).

Viable seed production differed between year (*F*- test $P = 0.013$, Table 1a), but did not differ between scrub type (*F*- test $P = 0.525$, Table 1a). In 1997, as reported above, no viable seeds were produced while in 1998 the viable seed production was of 19.33 ± 3.2842 SE, $n = 100$ plants.

Breeding structure and reproductive potential

Floral synchrony did not differ between scrub types or between years (*F*- test $P = 0.69$ and $P = 0.18$, respectively, Table 2a), but the interaction of both factors was significant (*F*- test $P = 0.006$). Multiple comparisons (Tukey-Kramer test ($P < 0.05$) showed that floral synchrony was lowest in the dense scrub in 1998 (0.31 ± 0.014 SE, $n = 50$ plants) than in the other three combinations of scrub type and year which did not differ among them (0.35 ± 0.003 SE, $n = 150$ plants). The range of the synchrony index values was similar in both scrub types and years and varied between 0.12 and 0.55. As expected, spacing among reproductive neighbors was also lower in the dense than in the diffuse scrub (4.63 m ± 0.26 SE vs. 13.01 m ± 1.63 SE, $n = 50$ plants in each, *F*- test $P < 0.0001$, Table 2b). However, the frequency distribution of spacing among reproductive neighbors differed markedly between scrub types (Figure 2, $\chi^2_{\mathrm{d.f.}\ 5} = 13.813$, $P = 0.0169$) indicating that the comparison of the effects of scrub-type and mean plant spacing on fitness components (as the ANOVA approach does) are far less appropriate than the analysis of the effect of plant spacing on plant fitness through the regression approach reported below.

The regression models of the fitness components on spacing between adults and flowering synchrony were significant, except for the effects of predation in the diffuse scrub in 1998 and total seed set (Table 3). In 1997, the harvested seeds did not germinate, therefore the reproductive efficiency was nil (Fig. 1), and we could not carry out the regression analysis with this variable (Table 3).

The variance explained by the regression models ranged from 2% in the case of the numbers of flowers, up to 68% in the case of predation in the diffuse scrub in 1997 (Table 3). In most cases only one explanatory variable was significant (Table 3). The spacing among reproductive neighbors was significant for the numbers of flowers and fruit set (in both years) but explained a small proportion of the variance (Table 3), while flowering synchrony was significant on its effect on predation (except for the diffuse scrub in 1998). Only in two cases were both explanatory variables significant (reproductive efficiency 1998 and viable seed production 1998). In these cases, the coefficients of partial regression (-0.16 and -0.08 for spacing among reproductive neighbors and 0.57 and 0.41 for flowering synchrony, in the reproductive efficiency and the viable seed production model respectively) showed that synchrony had the largest effect on reproductive efficiency and viable seed production.

The regression coefficients showed that as plant spacing increased, the number of flowers and fruits decreased (Table 3) indicating that, as compared with more isolated plants, plants growing in more clumped groups have more flowers and a higher proportion of flowers are successfully pollinated. The coefficients of regression also showed that the most synchronous individuals had higher reproductive efficiency than the less synchronous ones; but also, they had higher predation (Table 3). These results indicate that flowering synchrony increased production of viable seeds, but also increased damage by predation of flowers and fruits.

DISCUSSION

Flourensia cernua produced a high number of flowers per individual showing a relatively large reproductive potential (i.e., more than 5,000 ovules per individual per year). However, the female fertility for both years was very low (0 to 22 viable seeds in average per reproductive adult). Four main processes reduced the reproductive potential of the individuals: 1) predispersal seed predation (between 11% and 40% of initial flowers were damaged depending on the year and scrub type), 2) failure to be pollinated (between 22% and 28% of non damaged flowers depending on the year), 3) fruit abortion (ca. 90% of the initiated fruits were aborted independently of year and scrub type), and 4) low seed germinability (between 0 and 10% of seeds germinated depending on the year).

The curculionid *Smicronyx profusus* limited the production of viable seeds in two ways: it decreased the number of ovules available for pollination and it consumed fruits during the ripening phase. The higher predation in the diffuse scrubs than in the dense scrub could be related to a preference of the curculionid for this habitat, and/or to a satiation of the seed predators in the dense scrubs (e. g., Janzen, 1971; 1976; Platt, Hill and Clark, 1974). Predispersal seed predation can affect plant recruitment (Louda, 1982; 1983) and, in the case of *F. cernua*, this effect varies between years and scrub types.

The recorded fruit set in *F. cernua* was relatively high (an average of 72% of non predated flowers set fruits each year), suggesting that pollen and/or compatible mate availability were high enough to allow fertilization of a large proportion of the ovules. Nevertheless, pollination success varied among years, suggesting that in some years pollen limitation can be a restrictive factor for fruit initiation (i.e., in 1998 the failures in pollination were almost 20% higher than in 1997).

Despite the large number of fruits that were initiated in *F. cernua*, the production of seeds was very low as a consequence of the large levels of fruit abortion. Resource limitation and/or high genetic load could explain the fruit abortion (Wiens, 1984; Charlesworth, 1989). In 31 perennial species, predominantly cross-pollinated, Wiens (1984) found high rates of fruit abortion (52.3% ± 18.9 SE). In *F. cernua* the rates of fruit abortion were even larger (90.45% ±

1.97 SE). Fruit abortion could be predominantly caused or exacerbated by by environmental factors such as water availability, photoperiod, extreme temperatures and the position of the flower in the inflorescence or on the plant (Schemske et al., 1978; Stephenson, 1981; Gutterman, 1993; Diggler, 1995). In this sense, it has been found that the number of seeds ripening in *F. cernua* is negatively correlated with the minimum temperatures registered in the post-flowering season (Valencia-Díaz and Montaña, unpub.).

Finally the production of viable seeds in *F. cernua* was limited by low germinability of seeds (ca. 10% of filled seeds. Indeed the seed germinability in *F. cernua* is extremely low when compared with the germinability of other 112 species of the Asteraceae (Valencia-Díaz and Montaña, 2003). These authors suggest that the low germinability of seeds this species could be related with inbreeding depression, which is related with genetic load.

In summary, our results suggest that the main factors limiting the fertility of *F. cernua* (i. e. production of viable seeds) are: 1) predispersal fruit predation which varies between scrub types and years, 2) low pollen availability during the pollination phase which varies mainly between years, 3) high fruit abortion rate and, 4) low seed germinability.

Breeding structure and reproductive potential

The breeding structure varied between scrub types: in the diffuse scrub reproductive neighbors were more distant among them but they flowered more synchronously than in dense scrub. Flowering synchrony promotes pollen exchange, and consequently, increases cross-pollination rates (Bawa, 1983; O'Neil, 1997). The latter may be particularly important in *F. cernua* in which a self-incompatibility system, probably similar to that found in other members of the Asteraceae, has been found by Ferrer et al. (chapter two). Higher reproductive efficiency in the most synchronously flowering individuals may also be influenced by their higher chance of obtaining compatible mates when more plants (i.e. S-genotypes) are flowering simultaneously.

Similar results were found by Waser (1978) in two orchids and by O'Neil (1997) in a Lythraceae. We believe that the increase in fitness related to an increase in the synchrony index could lead to a directional phenotypic selection in phenological traits that promote flowering synchrony in *F. cernua*, but this directional selection could be counterbalanced by an increase in flower and fruit predation.

The higher potential fertility expected in dense clumps of plants (i.e., Allison, 1990; Arista and Talavera, 1996) could also be counterbalanced by the higher relatedness (and thus higher biparental inbreeding depression) between plants of these scrubs. Biparental inbreeding depression (expressed as high abortion and low germinability) may occur more frequently in plants growing in dense clumps because seedling establishment tends to occur in close proximity to the mother plant (Mauchamp *et al.*, 1993).

In summary, lower spacing among reproductive neighbors and high flowering synchrony had a positive effect on reproductive efficiency. Although reproductive efficiency (i.e. viable seeds / initial flowers) was similar in both scrub types, the higher density of reproductive adults could lead to a large fecundity (viable seeds / unit area) in the dense scrub. Besides given the higher water availability in the dense scrub compared with the diffuse scrub could favor the recruitment of new seedlings in these sites (Montaña *et al.*, 1990; Mauchamp *et al.*, 1993) giving rise to a positive feedback towards increased plant density, at least until density-dependent factors (e.g. intraspecific competition, diseases, herbivory, Mauchamp et al. 1993) take lead in the regulation of population growth.

The low production of viable seeds is an important factor that limit fitness in species lacking vegetative reproduction. Besides factors such as predispersal fruit predation, low pollen availability, high fruit abortion, and low germinability, the temporal and the spacial arrangement of reproductive adults limited female fitness of *F. cernua*. In these sense mate availability (determined by plant spacing and flowering synchrony) could influence the plant fitness of species, which like *F. cernua* have a partial self-incompatible system. In partial self-incompatible species natural selection may favor phenological traits that promote flowering synchrony of individuals, or mechanisms that promote lower spacing among individuals. These hypothesys could be tested with studies similar to the present and will improve our understanding of the evolution of fenological traits and the evolution of partial self-incompatibility.

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Table 1. Results of repeated-measures ANOVAS for six female fitness components of *Flourensia cernua*. In each ANOVA the independent variables of fixed effects were scrub type (dense vs. diffuse) and year (1997 vs. 1998) and independent variables of random effects were sub-plot for all female fitness component and individual for the last six of them. Nested factors are given between brackets. A pair of factor names separated by an × indicate the statistical interaction between them. The fitness components showed in **(a)** were analyzed with a different model (i.e. it does not include the effect of individual plants) as those used to analyze the fitness components showed in **(b)**.

a)

Flowers	df	MS effect	MS error	*F*	*P*	R^2
Scrub type	1, 8	798689	135166208	0.006	0.941	0.001
Sub-plot [scrub type]	8, 90	135166208	20796066	6.500	<0.001	0.893
Year	1, 8	2390078	1083427	2.206	0.176	0.002
Scrub type × year	1, 8	14738	1083427	0.014	0.910	0.000
Sub-plot [scrub type] × year	8, 90	1083427	1308818	0.828	0.580	0.007
Viable seeds produced						
Scrub type	1, 8	2262.660	5116.322	0.442	0.525	0.000
Sub-plot [scrub type]	8, 90	5116.320	1647.448	3.106	0.004	0.020
Year	1, 8	52407.770	5116.322	10.243	0.013	0.033
Scrub type × year	1, 8	2262.660	5116.322	0.442	0.525	0.000
Sub-plot [scrub type] × year	8, 90	5116.320	1647.448	3.106	0.004	0.020

b)

Predation	df	MS effect	MS error	F	P	R^2
Scrub type	1, 8	5.392	0.903	5.972	0.040	0.035
Sub-plot [scrub type]	8, 90	0.903	0.446	2.025	0.052	0.047
Individual [sub-plot, scrub type]	90, 400	0.446	0.102	4.359	<0.001	0.263
Year	1, 8	18.370	0.261	70.345	<0.001	0.120
Scrub type × Year	1, 8	3.430	0.261	13.134	0.007	0.022
Sub-plot [scrub type] × year	8, 90	0.261	0.403	0.648	0.736	0.014
Individual [sub-plot, scrub type] × year	90, 400	0.403	0.099	4.071	<0.001	0.238
Fruit set						
Scrub type	1, 8	1.943	1.292	1.504	0.255	0.013
Sub-plot [scrub type]	8, 90	1.292	0.691	1.871	0.074	0.070
Individual [sub-plot, scrub type]	90, 400	0.691	0.102	6.771	<0.001	0.420
Year	1, 8	1.512	0.251	6.034	0.040	0.010
Scrub type × Year	1, 8	0.006	0.251	0.023	0.882	<0.001
Sub-plot [scrub type] × year	8, 90	0.251	0.393	0.637	0.745	0.014
Individual [sub-plot, scrub type] × year	90, 400	0.393	0.087	4.538	<0.001	0.239
Seed set						
Scrub type	1, 8	0.008	0.685	0.011	0.918	<0.001
Sub-plot [scrub type]	8, 90	0.685	0.269	2.551	0.015	0.079
Individual [sub-plot, scrub type]	90, 400	0.269	0.017	15.925	<0.001	0.349
Year	1, 8	0.853	0.827	1.032	0.340	0.012
Scrub type × Year	1, 8	0.010	0.827	0.012	0.916	<0.001
Sub-plot [scrub type] × year	8, 90	0.827	0.265	3.122	0.004	0.095
Individual [sub-plot, scrub type] × year	90, 400	0.265	0.021	12.772	<0.001	0.344
Reproductive efficiency						
Scrub type	1, 8	0.009	0.010	0.944	0.360	0.006
Sub-plot [scrub type]	8, 90	0.010	0.004	2.656	0.012	0.052
Individual [sub-plot, scrub type]	90, 400	0.004	0.001	2.963	<0.001	0.219
Year	1, 8	0.185	0.010	18.560	0.003	0.120
Scrub type × Year	1, 8	0.009	0.010	0.944	0.360	0.006
Sub-plot [scrub type] × year	8, 90	0.010	0.004	2.656	0.012	0.052
Individual [sub-plot, scrub type] × year	90, 400	0.004	0.001	2.963	<0.001	0.219

Table 2. a) Result of the repeated-measures ANOVA for the synchrony index of *Flourensia cernua* plants. Independent variables were scrub type (dense vs. diffuse) and year (1997 vs. 1998). **b)** Results of the nested ANOVA for the spacing among reproductive neighbors of *Flourensia cernua* plants. Independent factors were scrub type (dense vs. diffuse), sub-plots (five sub-plots nested in each scrub type) and individual (10 individuals nested in each sub-plot). The between year variability of spacing among reproductive neighbors could not be analyzed because reproductive neighbors were the same in both years. Nested factors are given in brackets. A pair of factor names separated by the symbol × indicate the statistical interaction between them.

a) Synchrony Index

Source	MS effect	MS error	*F*	*d.f.*	*P*	R^2
Scrub type	0.002	0.009	0.176	1, 8	0.686	0.0007
Sub-plot [scrub type]	0.009	0.021	0.429	8, 90	0.901	0.0313
Year	0.009	0.004	2.168	1, 8	0.179	0.0039
Scrub type × year	0.057	0.004	13.597	1, 8	0.006	0.0245
Sub-plot [scrub type] × year	0.004	0.003	1.540	8, 90	0.155	0.0144

b) Spacing among Reproductive Neighbors

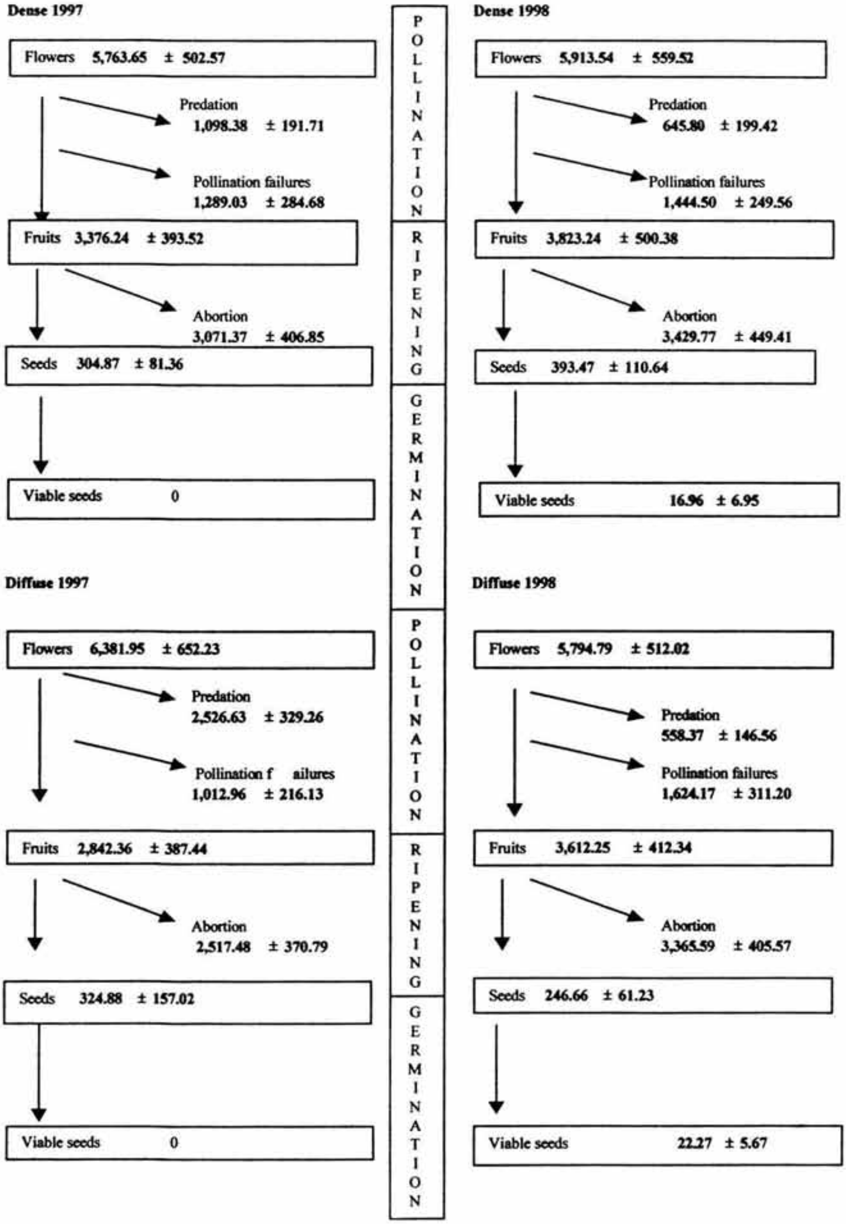
Source	MS effect	MS error	*F*	*d.f.*	*P*	R^2
Scrub type	3.640	29.119	23.277	1,90	<0.0001	0.377
Sub-plot [Scrub type]	4.212	4.212	26.933	8,90	<0.0001	0.008

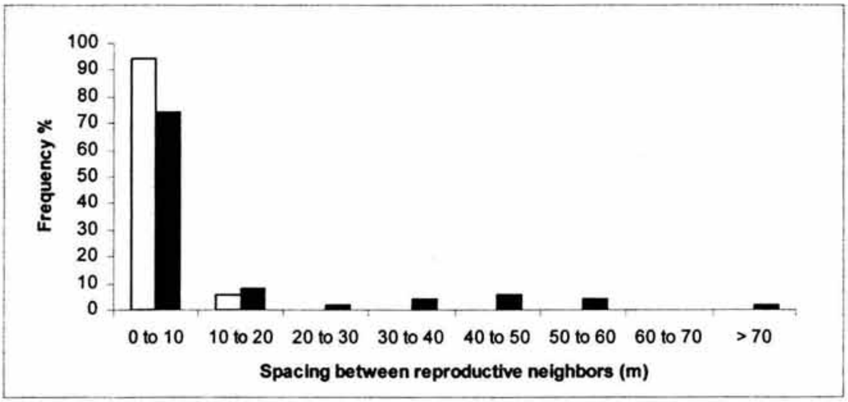
Table 3. Models and coefficients of the regressions between fitness components and flowering synchrony (FS) and spacing among reproductive neighbors (RN) for dense and diffuse scrubs in 1997 and 1998. All models were fitted by a forward stepwise procedure. Regression analyses of the number of flowers and the seed set were performed after pooling the data of both years and both scrub types as no differences between scrub types and years were revealed by the ANOVA (results shown in Table 1). Due to a similar reason the fruit set data of both scrub types were pooled to perform a separate regression for each year. In 1997 reproductive efficiency and production of viable seeds was nil and the regression analyses were not performed. The models in which non significant effect was found are not shown in this table.

		Model	R^2 *Adj.*	*F*	*df*	*P*
Flowers	Total	Y =0.017-0.185 (RN)	0.021	5.285	1,198	0.023
Predation	Dense 1997	Y = 0 +0.550 (FS)	0.397	33.963	1,49	<0.0001
Predation	Dense 1998	Y = 0+0.346 (FS)	0.166	10.982	1,49	<0.0001
Predation	Diffuse 1997	Y = 0 +1.088 (FS)	0.682	108.66	1,49	<0.0001
Fruit set	1997	Y = 0.673 –0.389 (RN)	0.0412	5.250	1,98	0.024
Fruit set	1998	Y = 0.745 –0.475 (RN)	0.074	8.858	1,98	0.004
Reproductive efficiency	1998	Y = 0 –0.007 (RN) + 0.015 (FS)	0.229	15.608	2,98	<0.0001
Viable seed produced	1998	Y = 0 – 0.0145 (RN) + 0.322 (FS)	0.187	12.472	2,98	<0.0001

Figure 1. Flow diagrams showing *Flourensia cernua* flower production and flower fate in dense and diffuse Chihuahuan Desert scrubs in two years (1997 and 1998). Mean values per plant ± SE, n = 50 plants, are shown. Per plant values were obtained after multiplying the fitness components by the total number of flowers produced per individual.

Figure 2. Frequency distribution of plants according to categories of spacing between reproductive neighbours (mean distances from the four nearest neighbours to the focal plant) from 50 plants in dense scrub (open bars) and 50 plants from diffuse scrub (filled bars). Data gathered for *Flourensia cernua* in the Southwestern Chihuahuan Desert.


Dense 1997
Flowers 5,763.65 ± 502.57
Predation 1,098.38 ± 191.71
Pollination failures 1,289.03 ± 284.68
Fruits 3,376.24 ± 393.52
Abortion 3,071.37 ± 406.85
Seeds 304.87 ± 81.36
Viable seeds 0
Dense 1998
Flowers 5,913.54 ± 559.52
Predation 645.80 ± 199.42
Pollination failures 1,444.50 ± 249.56
Fruits 3,823.24 ± 500.38
Abortion 3,429.77 ± 449.41
Seeds 393.47 ± 110.64
Viable seeds 16.96 ± 6.95
POLLINATION
RIPENING
GERMINATION
POLLINATION
RIPENING
GERMINATION
Diffuse 1997
Flowers 6,381.95 ± 652.23
Predation 2,526.63 ± 329.26
Pollination failures 1,012.96 ± 216.13
Fruits 2,842.36 ± 387.44
Abortion 2,517.48 ± 370.79
Seeds 324.88 ± 157.02
Viable seeds 0
Diffuse 1998
Flowers 5,794.79 ± 512.02
Predation 558.37 ± 146.56
Pollination failures 1,624.17 ± 311.20
Fruits 3,612.25 ± 412.34
Abortion 3,365.59 ± 405.57
Seeds 246.66 ± 61.23
Viable seeds 22.27 ± 5.67


Frequency %
100
90
80
70
60
50
40
30
20
10
0
0 to 10
10 to 20
20 to 30
30 to 40
40 to 50
50 to 60
60 to 70
> 70
Spacing between reproductive neighbors (m)

Ferrer et al. Partial self-incompatibility in *Flourensia cernua*

Breeding system and inbreeding depression in *Flourensia cernua* (Asteraceae): a case of partial self-incompatibility

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Abstract

We examine the contribution of inbreeding depression and self-incompatibility to low seed production and seed viability observed in *Flourensia cernua* using self-pollination, cross-pollination and open-pollination control treatments.

We found that 55% of plants were completely self-incompatible. Analysis of pollen tubes after self-pollination suggested the presence of a sporophytic self-incompatibility system. The remaining 45% of individuals, were partially self-incompatible and exhibited inbreeding depression for seed germinability. Seed set was higher after cross- pollination and did not differ between self and open-pollination treatments, but germinability was lower after self pollinatiton. Self-compatible individuals (those having a self-incompatibility index > 0.5) displayed larger fitness (production of viable seeds) than self-incompatible individuals. These results suggest that 1) S-allele diversity is low and 2) inbreeding depression (in addition to partial or full self-incompatibility) does contribute to reduce the production of viable seeds.

The partial breakdown of the incompatibility system, facilitated by a low S-allele diversity, is advantageous when population density of wind pollinated species is low by allowing more inbred mating and compensating for reduced pollen availability. Furthermore, the higher fitness of self-compatible individuals suggests that these lineages may carry less genetic load.

Key words: Breeding system, *Flourensia cernua,* inbreeding depression, partial self-incompatibility, population density, seed set limitations

INTRODUCTION

Genetic self-incompatibility systems are important in avoiding inbreeding in flowering plants (Barrett, 1998; De Nettancourt, 2001). By definition, self-incompatible species develop zero to very low numbers of seeds after self-pollination, due to the action of self-incompatible genes which retard the growth of pollen tubes when pollen and stigma express the same alleles at the S-locus. Two types of incompatibility systems have been described, gametophytic and sporophytic depending on the tissues (haploid or diploid) of the pollen grain where the proteins that trigger the incompatibility reaction have been produced. In the gametophytic incompatibility system the growth of pollen tubes is arrested in the stile if the haploid genotype of pollen shares an S-allele with the recipient plant (Barrett, 1998; De Nettancourt, 2001). In the sporophytic incompatibility system the pollen usually fails to hydrate and germinate on the stigma, if the diploid genotype of parent plant producing pollen shares an S-allele with the recipient plant (Barrett, 1998; De Nettancourt, 2001). Thus, because each individual may only mate with other individuals carrying S-alleles distinct from their own, the mean reproductive success of individuals increases with the number of S-alleles in the population.

Self-incompatible species often show relatively low seed set (Sutherland and Delph, 1984; Sutherland, 1986), a condition that depends upon the type of incompatibility system (gametophytic or sporophytic – with sporophytic being more limiting), the number of S-alleles in the population and the availability of suitable pollinators. Nevertheless, some predominantly outcrossing, self-compatible species also show low seed set. Among of the causes of the reduction in seed set in self-compatible, highly outcrossing species are low pollinator availability, or high rates of seed abortion in populations with high levels of genetic load (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). A reduction in female fertility caused by self-incompatibility is strictly a prezygotic mechanism (but see Richards, 1986, Sage et al., 1994) while one caused by inbreeding depression is strictly a post-zygotic phenomenon (Charlesworth and Charlesworth, 1987).

Though many species are believed to be strongly self-incompatible, the evolution from being predominantly self-incompatible to being predominantly self-compatible has probably also occurred many times (Igic and Kohn, 2001). The evolution from self incompatible to self compatible has been documented when individual fitness is limited by the availability of compatible pollen, regardless of whether this condition is due to a lack of pollinators (Berry and Calvo, 1989) or a low diversity of S (self-incompatibility) alleles (Byers, 1995; Reinartz and Les, 1994). Similarly, self-compatibility might be an advantageous trait if seed dispersal is spatially limited (e. g., dispersal relying on gravity) and/or if related individuals occur close together, thus allowing for frequent inbred, biparental mating (Levin, 1986, 1996). When the fitness of progeny produced by selfing is higher than that of progeny produced by outcrossing (i.e. when inbreeding depression is low) the gene (or genes) responsible for self-compatibility increases its frequency, thus facilitating the evolutionary transition from self-incompatibility to self-compatibility (Lande and Schemske, 1985; Levin, 1986, 1996). Genetic models suggest that levels of inbreeding depression higher than 0.5 typically inhibit the evolution of self-compatibility (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1990).

In addition to documenting a transition from complete self-incompatibility to full self-compatibility, in recent years, an increasing number of studies have documented the existence of partial self-incompatibility in natural populations of plants. Partially self-incompatible species are thoses in which there is quantitative variation among individuals in the strength of self-incompatibility (individuals from strongly to weakly self-incompatible) or variation in the expression of self-incompatibility associated with changes in temperature, floral age or flowering time (Levin, 1996). Many studies have reported partial self-incompatibility in the Asteraceae family (i. e., Berry and Calvo, 1989; De Mauro, 1991; Byers and Meagher, 1992; Luitjen et al., 1996; Young et al., 2000) although the factors selecting for partial self-incompatibility and its evolutionary importance are still poorly understood (Reinartz and Les, 1994; Hiscock, 2000). In particular, it is not clear wether partial self-incompatibility is most commonly a transitional stage in the progression from full self-incompatibility to self-compatibility, or it is an evolutionarily stable mating strategy.

Flourensia cernua (Asteraceae, Heliantheae) is a shrub characteristic of the Chihuahuan Desert (MacMahon, 1989). It has a low seed set (8.79% ± 0.19 SE of ovules produced seeds), but one within the range of values previously reported for other self -incompatible plants (Sutherland and Delph, 1984). It also shows very low rates of seed germination (ca 9%), less than the mean reported for 112 species of plants within the Asteraceae (74. 07%± 2.79 SE Valencia - Díaz and Montaña, 2003). These observations lead to the hypothesis that a sporophytic, multiallelic, self-incompatibility system might exist in *F. cernua*, similar to systems that have been previously described in the family Asteraceae (Richards, 1986; Hiscock, 2000).

This study tests the hypothesis that the low fertility observed in *F. cernua* is due to the existence of a self-incompatibility system and/or to the effects of inbreeding depression. Additionally, it tests the hypothesis that the expression of both self-incompatibility and inbreeding depression may vary with population density (Farris and Mitton, 1984, Allison, 1990, Arista and Talavera, 1996). To this end, seed set and germinability were estimated for three pollination treatments (self-pollination, cross- pollination, and an open- pollinated control) in two scrub types with contrasting population densities (diffuse, low density scrub and dense, high density scrub). The results of this experiment were used to evaluate: 1) the presence of self-incompatibility, 2) the effect of pollen origin on seed set and germinability, 3) the number of viable seeds produced by the self-compatible and the self-incompatible individuals, and 4) the spatial variability of self-incompatibility and inbreeding depression indices.

MATERIALS AND METHODS

Species and study site

Flourensia cernua is a perennial shrub with hermaphroditic protandrous flowers, which are wind-pollinated. It is considered as an indicator plant species of the Chihuahuan Desert (MacMahon, 1989). It produces flowers at the end of autumn (October-November) and fruits, the single-seeded achenes typical of the Asteraceae, during the winter (December-February) (Dillon, 1984). We have found low female fertility (0 to 22 viable seeds resulted from approximately 5,000 flowers per individual, per reproductive season), a reduction in reproductive potential by fruit predation (approximately 25% of the initial flowers), and failure pollination (approximately 25% of the non predated flowers). Additionally, a large number of aborted fruits (approximately 90% of all fruits) and low seed germination rates (less than 10% of all seeds were viable) contributed to the reduced reproductive potential. This low production of viable seeds may have been due to a limited availability of potential mates and/or inbreeding depression in mating between closely related individuals.

The study was undertaken at the Mapimí Biosphere Reserve (26° 40' N and 103° 40' W; 1,100 m above sea level; 264 mm average annual precipitation, 72% falling between June and September; Cornet, 1988), in the southwestern portion of the Chihuahuan desert. In this region, *F. cernua* is found in two contrasting vegetation types: 1) diffuse, low density scrub and 2) dense, highly clumped scrub. The dense scrub do not differ from diffuse scrub in general climatic or edaphic characteristics, except for a slightly higher clay content in the soil and higher water availability due to runoff rain-water redistribution (Cornet et al., 1992, Montaña, 1992, Galle et al., 2001). They are contiguous with diffuse scrubs, which inhabit terrain upslope from dense scrub (ca. 1.5 km). Density of reproductive adults of *F. cernua* is 81 individuals/ha ± 0.5 SE (measured in five ≈ 1.5-ha permanent plots used for long term demographic studies). The dominant species (according to cover values) are *Larrea tridentata*, *Opuntia rastrera* and *Jatropha dioica*. The dense scrubs have slight slopes (<1.5%); and density of reproductive adults is 878 individuals/ha ± 35 SE (measured in five ≈ 0.4-ha permanent plots used for long term demographic studies). The dominant species (according to cover values) are *Prosopis glandulosa* var. *torreyana*, *Hilaria mutica* and *F. cernua*. All reported permanent plots were randomly selected in each scrub type based on the vegetation map of the Mapimí Biosphere Reserve (Montaña, 1988) over an area of 20 km^2 (7 km^{2}of dense scrub and 13 km^2 of diffuse scrub).

Pollination experiment

Pollination experiments were carried out in one study sub-plot located arround the centre of the permanent plots of demographic survey. In October 1998, ten reproductive adults per sub-plot were tagged in five diffuse and five dense scrubs. Plants were fumigated with insecticide (Deltametrin; 12.5 mg/l) two times per week from October 15th until the date of pollination (6 to 25 November) to prevent flower predation by flies and beetles. Fifteen stems were randomly chosen from each of the 100 tagged individuals, and then five stems from each group of fifteen stems were randomly assigned to one of three experimental treatments. Treatments consisted of: 1) self-pollination of flowers using pollen from a different stem of the same plant, 2) cross-pollination between a female recipient and 1-5 pollen donors selected from synchronously blooming plants at least 250 m away, and 3) a open-pollinated control in which no pollen was applied and the inflorescence was left uncovered during the entire flowering season, and then bagged at flower senescence. Following the hand-pollination treatments, all stems were wrapped in fine mesh bags to prevent contamination by exogenous pollen. For hand pollinations, every other day pollen was collected and applied with a soft-bristle brush in every focal individual during the female phase of inflorescence. On each stem the number of experimental flowers was recorded every other day and, at the end of the flowering season, the bags were left on all experimental stems to prevent seed loss and predation.

The 15 experimental stems from each plant were collected in February, 1999, transported to the laboratory, and filled seeds were stored in paper bags at ambient temperature. A filled seed was determined to be an achene that did not break after being squeezed gently with forceps and that contained an embryo occupying at least three-quarters of the fruit. Preliminary assays (N = 500) demonstrated that achenes that did not resist gentle squeezing or that did not contain an embryo occupying more than three-quarters of the achene were not viable. During the months of July and August, 1999, all filled seeds were placed in an environmental chamber to germinate on a moistened cotton substrate (12 hours light at 26°C: 12 hours darkness at 16°C; 60% relative humidity). After 25 days (when most of viable seeds were germinated according to Mauchamp 1992 and Valencia-Díaz and Montaña 2003), the number of germinated seeds was counted.

Self-incompatibility

Self-incompatibility index (SI = seed set after self-pollination/seed set after cross-pollination) was calculated for each experimental plant. Individuals were then grouped by their index values into the following four categories (modified from Zapata and Arroyo, 1978): 1) incompatible (SI = 0.000), 2) slightly compatible (0.000 < SI < 0.149), 3) compatible (0.150 < SI < 0.490), and 4) fully compatible (SI > 0.500). Frequency distributions of the categories of self-incompatibility, in the two scrub types were compared using a χ^2 test (Sokal and Rohlf, 1995).

The self-incompatibility index does not allow the discrimination between a prezygotic (i.e. a truly expression of self incompatibility) and a postzygotic mechanism (i.e. the interruption of seed development due to the expression of inbreeding deepression at very early stages of seed development). The expression of a prezygotic mechanism was assessed by careful microscopic observation of styles and stigmas of flowers previously subjected to different hand pollination treatments.

For this purpose, five additional individuals from the dense scrub and five individuals from the diffuse scrub were hand pollinated. For each individual plant, five flower heads were randomly assigned to a self-pollination treatment and another five were randomly assigned to a cross- pollination treatment. At 6, 9, 12, 18 and 24 hours after pollination, three flowers were collected from each flower head. These flowers were fixed (6:3:1 solution of formol, glacial acetic acid, and ethanol) for an hour, and then transferred to a solution of 0.5% aniline-blue, in K_3PO_4 at 4°C for 30 days. At the end of this time, samples were rinsed, transferred to 20% alcohol for 12 hours, and mounted on slides for observation (Olympus BHA microscope with vertical fluorescent lamp). Styles were carefully examined for the presence of callous body, an indicator of interrupted pollen tube growth. Such interrupted growth is considered evidence of a pre-zygotic, self-incompatibility system and in the Asteraceae appears as an area of darkened stain around the stigmatic papillae in association with pollen grains/tubes (cf., Hiscock 2000).

Pollen origin, plant fitness and population density

Fitness measures (calculated on a per stem basis) for statistical analyses were: 1) seed set, expressed as the number of filled seeds produced per ovule and 2) germinability, expressed as the number of germinated seeds per filled seed produced. Only plants that produced fruits in at least one of the three pollination treatments (30 plants in the dense scrub and 31 plants in the diffuse scrub) were included in the analysis. The sampling unit for seed set and germinability was the number of pollinated stems per individual ($N = 5$). Final sample sizes were of $N = 150$ stems for dense scrub and $N = 155$ stems for diffuse scrub.

To evaluate the variation in fitness components associated with population density and pollen origin a repeated-measures ANOVAs (implemented through a MANOVAR procedure, see Potvin, *et al.*, 1990, also called MANOVA procedure Von Ende, 1993) were carried out for the response variables seed set and germinability. Fixed factors were pollination treatment (repetition factor) and scrub type. Sub-plots were nested within scrub type and individuals were nested within sub-plots and scrub type, both being considered random factors. The original data for seed set and germinability were subjected to an angular transformation because proportion and percentage data were not distributed normally (Sokal and Rohlf, 1995).

Plant fitness and self-incompatibility

To assess the influence of self-incompatibility on plant fitness the variance in the number of viable seeds obtained in the open pollinated (control) treatment as a function of the self-incompatibility degree measured through the SI was analyzed using a unifactorial ANOVA (Sokal and Rohlf, 1995). The degree of self-incompatibility was a categorical variable with the four levels as defined above: 1) incompatible, 2) slightly compatible, 3) compatible, and 4) fully compatible. The dependent variable expressing plant fitness in a per plant basis was viable seed production from the open-pollinated (control) treatment estimated by the triple product of seed set by germinability and by the total number of flowers per plant. Preliminary analyses using scrub type, degree of self-incompatibility, and their interaction as independent variables showed that neither scrub type nor the interaction between scrub type and self-incompatibility degree were significant and the final ANOVA was conducted using only self-incompatibility degree.

Inbreeding depression, population density and self-incompatibility degree

Inbreeding depression was calculated only for plants that produced seeds after both self and cross- pollination treatment (*i. e.* for self-compatible plants which comprised 45 out of the 100 experimental plants). For these plants the inbreeding depression index of germinability was calculated according to the formula: $\delta = 1 - (w_i / w_o)$; where δ is the inbreeding depression index, w_i is the fitness of progeny obtained from self-pollination, and w_o is the fitness of progeny resulting from cross-pollination (Lande and Schemske, 1985). Germinability was estimated by dividing the number of germinated seeds by the total number of filled seeds from the five stems.

Variation in inbreeding depression index of germinability as a function of scrub type (diffuse and dense) were analyzed by a nested ANOVA. Scrub type was a fixed effect, and sub-plot was a random effect nested within scrub type.

The correlation between inbreeding depression in germinability (which reflects a postzygotic interruption of seed development) and the self-incompatibility (which reflects a prezygotic interruption of seed development) index was assessed through a Spearman correlation (Sokal and Rohlf, 1995). The evolution from self-incompatibility to self-compatibility requires that a progressive purging of genetic load pushes down inbreeding depression. A negative correlation between inbreeding depression and the self-incompatibility index would suggest the feasibility of the evolution from self-incompatibility to self-compatibility, while a positive correlation would suggest that this evolution is not possible. The lack of correlation would not allow to predict the direction in the evolution of a current mixed mating system. All statistical analysis were performed with STATISTICA (Statsoft Inc., 1998)

RESULTS

Presence of self-incompatibility

We found that 55% of the individuals produced exclusively empty fruits after self-pollination, but did produce fruits after cross pollination. Such a result suggests that a pre-zygotic mechanism was responsible for the absence of seed production. The remaining 45% of the individuals were self-compatible to some degree, producing a variable number of seeds after self-pollination. In total, 9% of the individuals were slightly compatible, 16% were compatible, and 20% were fully compatible. Distribution frequencies of the four self-incompatibility categories did not differ between scrub types ($\chi^2_{df\,5} = 3.5369$, $P= 0.3160$, Fig. 1). Thus in both scrub types nearly half of the population was self-incompatible, and the remaining individuals expressed variable levels of partial breakdown of the self-incompatibility system.

Provided additional evidence from a microscopy work. Stigmas of self-pollinated flower from strongly self-incompatible individuals were found to develop highly dense areas of callous deposition around areas of very short pollen tube growth through the papillary cells on the stigmatic surface. In contrast, there was an absence, or in some cases, only a reduction in the number of callous bodies, found on the stigmatic surfaces of cross- pollinated pistils suggesting that these crosses were either fully compatible or partially or completely incompatible. Furthermore, there was no evidence of pollen tubes in the stylar tissue from strongly self-incompatible individuals.

Pollen origin, plant fitness and population density

Pollen origin and density affected seed set and germinability in *F. cernua* (Table 1). Cross-pollination resulted in seed set that was more than twice the value obtained for the self-pollination treatment in both scrub types (Tukey-Kramer test, $P \leq 0.0001$, Table 2). In addition, in both scrub types seed set from the cross pollination treatment was significantly higher than that of open pollinated flowers (Tukey-Kramer test, $P \leq 0.0001$, Table 2). Seed set from cross pollination was 46% higher in the diffuse scrub than in the dense scrub (Tukey-Krammer test, $P \leq 0.0001$, Table 2), suggesting that seed set is limited by pollen availability, particularly in the diffuse scrub.

The germinability was more than three times higher after cross than after self-pollination in the dense scrub (Tukey-Krammer test, $P \leq 0.0001$, Table 2), but, interestingly, the proportion of seeds that germinated from the dense scrub was larger after open-pollination control than after the cross-pollination treatment (1.8 times larger Table 2). The germinability was 2.7 times higher for cross- than for self-pollination (Tukey-Krammer test, $P \leq 0.0001$, Table 2), and 1.92 times higher than the germinability of seeds from open-pollination treatment in the diffuse scrub (Table 2). These results indicate that seed set and germinability of hand cross-pollinated flowers was generally highest. Open- pollinated control and hand self-pollinated flowers had similar levels of seed set but open-pollinated flowers had higher rates of seed germination, especially in the dense scrub.

The interaction effect between individual and pollination type was significant for both seed set and germinability (Table 1). This suggests that the mother plant may affect the fitness of their progeny through its self-incompatibility degree or genetic load.

Plant fitness and self-incompatibility degree

The degree of self-incompatibility had a significant effect on the production of viable seeds (Table 3 and Fig. 2). Fully compatible plants and compatible plants produced 2.25 and 2.14 times more viable seeds after open pollination respectively, than self-incompatible individuals (Fig. 2).

Inbreeding depression, population density and self-incompatibility degree

Only 45 out of the 100 individuals chosen for this experiment were found to produce seeds in both the self- and cross-pollination treatments. The δ of germinability (inbreeding depression index of germinability) were calculated for those plants and compared between scrub types to assess the effect of population density on the variation of inbreeding depression. There were no differences in δ of germinability between scrub types, means were 0.4303 ± 0.0372 SE, n= 20 in the dense scrub, and 0.1258 ± 0.0289, n = 25 in the diffuse scrub ($F_{1,8} = 2.0347$, $P = 0.1916$, Table 4).

The δ of germinability did not correlate with the self incompatibility index ($r = 0.0995$, $t = 0.6292$, $P = 0.5325$). However, the δ of germinability (*i. e.* inbreeding depression index) were in some cases negative (Fig. 3), suggesting that purging of genetic load is taking place in some individuals as a result of increased selfing.

DISCUSSION

In this study we found that the low fertility observed in *F. cernua* may be partially explained by the existence of a self-incompatibility system, and by the effects of inbreeding depression. In particular, we found that more seeds were produced following cross-pollination than following either self-pollination or the open-pollinated control and that this effect was larger in the diffuse than the dense scrub. Furthermore, the seeds produced after cross-pollination had higher germinability, i. e. higher viability, than those seeds of self- or open-pollinated flowers in the diffuse scrub, but a lower germinability than open-pollinated flowers in the dense scrub. These data strongly suggest that reproductive success in *F. cernua* is compromised by both low pollen availability (higher seed set after hand cross-pollination than in the open-pollinated control) and quality (higher germinability after hand cross-pollination, than in the self - pollination or in the open-pollinated control) in the diffuse scrub, while reproductive success is compromised mainly by pollen quality (higher germinability after open-pollinated control than cross- or self-pollination treatments) in the dense scrub.

Role of Self Incompatibility in plant fitness

Our data suggest that *F. cernua* probably has the same multi-allelic sporophytic self incompatibility system than that found in other members of the Asteraceae. The expression of self-incompatibility has been shown to vary according to diverse factors, such as the expression of dominance in *S* alleles, the expression of dominance in modifier genes affecting self-incompatibility, pollen origin (allogamous, exogamous, or mixtures of both), temperature, flower age, and the presence of developing fruits (Levin, 1996; Vogler et al., 1998; Stephenson et al., 2000; de Nettancourt, 2001; and references therein). Hiscock (2000) found that self-pollination in *Senecio squalidus*, a partially self- incompatible species, is possibly due to the presence of a cryptic, gametophytic system of self incompatibility operating in addition to a breakdown in sporophytic, self incompatibility in the species. To discern the physiological basis of pseudo self-incompatibility in *F. cernua* would require detailed reciprocal crossing experiments and microscope work which were outside the scope of this study and more difficult to perform in woody species because of the time needed to grow progeny to flowering.

In the sporophytic self-incompatibility systems, the incompatibility reaction occurs with geitonogamous pollination, or with cross-pollination, when the sporophyte producing pollen shares the same S-phenotype as the recipient plant (Richards, 1986; De Nettancourt, 2001). Geitonogamy is possible in *F. cernua* because male and female phases typically overlap within a flower head and between flower heads on different stems of the same plant, despite a temporal separation of two days between male and female phases within a flower (protandry). We found the same levels of seed set after the self-pollination treatment and the open pollinated control, but higher seed germination in open than self-pollinated flowers (Table 1), suggests that open-pollinated seed set is limited by the sharing of *S*-phenotypes between pollen donors and recipients but that the pollen that achieves fertilization is usually derived from cross-fertilization. These findings suggest that populations of *F. cernua* may have few *S*-alleles.

If large amounts of self or incompatible pollen arrive on stigmas of open- pollinated flowers of *F. cernua* it is possible that high levels of incompatible pollination would interfere with the germination of cross-pollen. Studies have shown that in species with sporophytic self-incompatibility, the deposition of incompatible pollen changes the physiological characteristics of the papillar cells on the stigmatic surface so that if cross-pollen is deposited onto a papillae after self-pollen it will be unable to germinate and grow (e. g. Silander and Primack, 1978; Garwood and Horvitz, 1985; Galen et al., 1989; Broyles and Wyatt, 1993). Given that we found evidence that a high proportion of pollen arriving to stigmas in *F. cernua* appears to be incompatible, this may be another factor limiting fruit set in the species.

Partial self-incompatibility

Many species in the Asteraceae possess a sporophytic, multi-allelic system of self-incompatibility (Richards, 1986). However, a partial breakdown of this system has also been reported for several species in the family (i. e., Berry and Calvo, 1989; De Mauro, 1991; Byers and Meagher, 1992; Luitjen et al., 1996; Young et al., 2000). A breakdown in self-incompatibility might have arisen as a response to reduced diversity of *S* alleles in small populations of *Aster furcatus* (Reinartz and Les, 1994), and possibly as a consequence of low seed set and/or low availability of pollinators in *Espeletia* (Berry and Calvo, 1989). In the genus

Espeletia the breakdown of the self-incompatibility system has been observed primarily in anemophilous species and this traits have been interpreted as a response to pollinator scarcity and adverse climatic conditions (Berry and Calvo, 1989). It is possible that breakdown of self-incompatibility in *F. cernua*, also an anemophilous species, might have evolved in a way similar to that postulated for *Espeletia*. However, this hypothesis can only be corroborated by an integrated study of the genus *Flourensia* as the one made by Berry and Calvo (1989)

In this study we found conclusive evidence that a breakdown in self-incompatibility system occurs in *F. cernua*. We also found evidence that seed set is limited by the quantity and quality of pollen in the diffuse scrub and mainly by quality of pollen in the dense scrub. Thus, it is possible that in both dense and diffuse scrub, the breakdown of the self-incompatibility system may have allowed for effective mating between plants that would not ordinarily be compatible (e. g., between close relatives or between plants sharing S-alleles; Levin, 1996). In this way, individuals expressing a partial breakdown of self-incompatibility might be able to compensate for potential limitations on female fitness at the population level due to the scarcity of potential mates.

Role of inbreeding depression in plant fitness

In addition to our evidence that reproduction is limited in *F. cernua* by the presence of self-incompatibility systems, we found evidence of strong inbreeding depression for an early of fitness trait, germinability.

Additionally, self fertilization, or mating among close relatives (biparental inbreeding), would affect the germination success of seeds as a consequence of inbreeding depression. In predominantly outcrossing plants, inbreeding depression is usually high regardless of whether the species is self-compatible (Husband and Schemske, 1996, and citations therein) or pseudo self-incompatible (Levin, 1996, and citations therein). If seedlings of *F. cernua* commonly establish near the mother plant (Mauchamp et al., 1993), then both geitonogamous and biparentally inbred matings probably occur. Therefore, inbreeding depression likely plays an important role in determining reproductive efficiency in *F. cernua*, as was suggested by the data on inbreeding depression for seed germination.

Although inbreeding depression is often severe at early stages in highly outcrossing species, (see reviews by Charlesworth and Charlesworth, 1987; Agren and Schemske, 1993; Husband and Schemske, 1996), the combination of low seed set with high inbreeding depression found in this study are unusually high. In this context, selection for self-compatibility will be prevented in the species. We believe that the presence of extensive variation in self-fertility and in inbreeding depression in natural populations of *F. cernua* explained the presence of a mixed mating system. The higher fecundity of self-compatible individuals, and the presence of some negative δ inbreeding depression of these plants (Fig. 3) suggests an evolutionary advantage for pseudo self-incompatibility in the species. However the pseudo self-incompatibility was not fixed because many individuals had not overcame the inbreeding depression associated with germinability (Fig. 3) and maybe with another traits not evaluated in this study.

Hamilton and Mitchell-Olds (1994) reported a significant interaction effect between pollination treatment and mother plant in *Arabis fecunda*, suggesting that inbreeding depression depended on the number of recessive, deleterious alleles carried by the mother (i. e. maternal genetic load). In *F. cernua* the interaction between individual and pollination type was significant, indicating that the mother's self-incompatibility degree or its genetic load did, in fact, influence the relative fitness of progeny derived from self- and cross-pollination. In the case of low seed set after self- pollination may be a consequence of differences in self-fertility, and the low seed set after cross pollination could be because of sharing of S-alleles between pollen donor and recipient. In the case of germinability and seed set in compatible plants the low seed set may be a consequence of genetic load and/or maternal effects.

Self-incompatibility breakdown and inbreeding depression

Despite high levels of inbreeding depression in slightly compatible and compatible plants in *F. cernua*, we suggest that self-incompatibility could evolve towards self-compatibility in this species. This suggestion is based upon the result of compatible and fully compatible plants having a higher fitness (i. e. they produce nearly twice of viable seeds per plant after open pollination). The increased fitness of fully compatible plants are mainly associated with the ability of producing seeds through self and cross-pollination. Moreover we found that fully compatible plants had negative δ of seed set (i. e. self-fertility index), which indicates that seeds derived from self-fertilization have increased fitness. In the absence of compatible cross-pollen, fully compatible individuals could produce equal or even more seeds through self-pollination. Therefore fitness differences between compatible and self-incompatible phenotypes could lead to a directional selection towards compatibility.

Self-incompatible species have shown to have bigger limitation in sexual reproduction due to pollen availability (Larson and Barrett, 2000). When the paucity of S-alleles limits reproduction or when pollen availability is also limiting reproduction, pseudo self-incompatibility may be selectively advantageous (Levin, 1996). Our results suggest that both a paucity of S-alleles and of low pollen availability had a negative effect on the seed set of *F. cernua*, and that this limiting effect is stronger in the diffuse than in the dense scrub. As was the case with *Taxus canadensis* (Allison, 1990), our results suggest that as a consequence of low population density in the diffuse scrub, low availability of compatible pollen limited seed set. For this reason, the presence of self-compatibility in *F. cernua* may be advantageous since it allows for an increased seed production in low density conditions. According to theoretical models, self-fertilization could be maintained by natural selection if inbreeding depression levels are lower than 0.5 (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1990), therefore pseudo self-incompatibility could be selected in some compatible plants since inbreeding depression on an early fitness component (*i. e.* δ of germinability) is lower than 0.5 and even negative (Fig. 3).

On the other hand, in the dense scrub, gravity dispersal in *F. cernua* seeds ensures that many offspring establishes near parent plants. Genetic structuring could limit reproduction of self-incompatible plants in these scrubs, because close relatives have a higher probability of sharing S-alleles. Self- compatibility in both scrub type could allow biparental inbreeding mating and assure seed production, but natural selection will tend to eliminate this characteristic since inbreeding depression in germinability could b be difficult to surmount.

The self-compatibility could favor the dynamic of colonization of *F. cernua* (see Mauchamp et al., 1993) due to the positive effects associated with the founding of new populations and/or the clumping of related individuals (Levin, 1996). Secondary dispersal of *F. cernua* seeds along ephemeral, surface water runoff courses might facilitate the colonization of dense scrubs from diffuse scrub. This possibility seems reasonable, since water-transported seeds will generally find suitable establishment microsites in dense scrubs (which are always located down slope from diffuse scrubs (Montaña et al., 1990). Self-compatible *F. cernua* individuals might colonize patches currently unoccupied by this species, leaving descendents either by selfing or outcrossing (even cross-pollination with individuals sharing the same S-alleles).

We propose that the breakdown of self-incompatibility system is maintained because it assures sexual reproduction despite the limitation in reproduction that this species confronts (both in seed set and germination) due to low pollen availability and a paucity of S-alleles. Self-compatibility confers *F. cernua* adults ecological advantages in both dense and diffuse scrubs because it compensates for limited pollen availability, allows fertilization among incompatible gametes (those of geitonogamous pollination or those who share S-alleles), and permits colonization of new patches. The pseudo self-incompatibility in *F. cernua* implies that a mixed mating system exists in this species, but the evolution of complete self- compatibility will occur only if inbreeding depression in all fitness components is surmounted.

Conclusions

In conclusion, *F. cernua* is a species that maintains a high degree of self-incompatibility, but that presents a mixed mating system in which some individuals produce progeny from self-pollination. As other self-incompatible species, it has a low seed set limited by pollen availability and a paucity of S-alleles. It also displays a high level of inbreeding depression associated with germinability (i. e. seeds derived from cross-pollination show higher germinability than seeds resulting from self-pollination). Self-compatibility confers advantages by compensating for low availability of compatible pollen (a situation given by low densities of reproductive adults in diffuse scrub and the presence of biparental, inbreeding mating). Partial self-incompatibility can be maintained in both scrub types because self-compatible individuals have fitness equal to, or higher than, the fitness associated with self-incompatible individuals and in some cases a decreased inbreeding depression of germinability of self-compatible plants.

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Table 1. Results of the repeated measures ANOVAs for seed set (total number of seeds/ovule) and germinability (number of viable seeds/total number of seeds) as a function of pollination treatment (self-pollination, cross-pollination and open-pollinated control) and scrub type (dense and diffuse). Data gathered from *Flourensia cernua* populations in the Southwestern Chihuahuan Desert.

Source		Seed set		Germinability	
	df	F	P	F	P
Between individuals					
Scrub type	1, 8	3.5313	0.0970	1.6536	0.2344
Sub-plot [scrub type]	8, 51	0.8275	0.5824	0.8795	0.5400
Individual [sub-plot, scrub type]	51, 244	6.1086	<0.0001	23.3138	<0.0001
Within infividuals					
Pollination treatment	2,16	46.6053	<0.0001	33.6119	<0.0001
Scrub type*Pollination treatment	2,16	4.5166	0.0292	8.2733	0.0003
Sub-plot [scrub type]*Pollination treatment	16,102	2.3225	0.0058	5.3409	<0.0001
Individual [sub-plot, scrub type]*Pollination treatment	102, 488	2.8066	<0.0001	6.9197	<0.0001

Table 2. Mean values and standard errors (in parentheses) for seed set (total number of seeds perovule) and germinability (number of viable seeds/total number of seeds) per each combination of scrub type (dense and diffuse) and pollination treatment (self-pollination, cross-pollination and open-pollinated control) in *Flourensia cernua* populations in the Southwestern Chihuahuan Desert. *N* = number of stems. Different lower case letters indicate significant differences between rows (Tukey-Krammer test, $P < 0.0001$). Different capital letters indicate significant differences between columns (Tukey-Krammer test, $P < 0.0001$).

	N	Self-pollination	Cross-pollination	Open-pollinated Control
1) Seed set				
Dense scrub	150	0.0726_{aA}	0.1818_{bB}	0.0760_{aA}
		(0.0139)	(0.0216)	(0.0079)
Diffuse scrub	155	0.0911_{aA}	0.2652_{cC}	0.0721_{aA}
		(0.0146)	(0.0227)	(0.0072)
2) Germinability				
Dense scrub	150	0.0429_{aA}	0.1394_{bB}	0.2503_{bcC}
		(0.067)	(0.0151)	(0.0228)
Diffuse scrub	155	0.0774_{aA}	0.2089_{cC}	0.1092_{bcB}
		(0.0071)	(0.0126)	(0.0168)

Table 3. Effect of self-incompatibility (incompatible, slightly compatible, compatible and fully compatible) on the number of viable seeds produced per plant after open-pollinated control. Data gathered from 61 *Flourensia cernua* plants in the Southwestern Chihuahuan Desert.

Source	df	SS	*F*	*P*
Self-incompatibility category	3	11348.10	2.8542	0.0452
Error	56	3975.96		

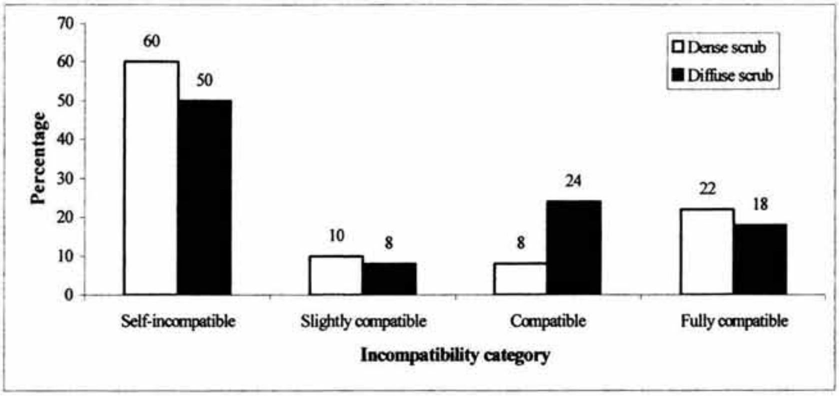
Table 4. Inbreeding depression index (δ) associated with germinability as a function of the scrub types (dense and diffuse) and sub-plot within scrub type. Data gathered from *Flourensia cernua* populations in the Southwestern Chihuahuan Desert.

Source	df	*F*	*P*
Scrub type	1,8	2.0347	0.1916
Sub-plot [Scrub type]	8,27	2.6906	0.0257

Figure 1. Frequency distribution of plants according to categories of self-incompatibility (SI = seed set after self-pollination /seed set after cross-pollination). Categories of self-incompatibility are defined as: self-incompatible SI = 0, slightly compatible 0 < SI < 0.149, compatible 0.15 < SI < 0.49, and fully compatible SI > 0.5. Data gathered for *Flourensia cernua* in the Southwestern Chihuahuan Desert. This hand pollination experiment involved 50 plants from dense scrubs and 50 plants from diffuse scrubs.

Figure 2. Mean value and standard error for the number of viable seeds produced by plants grouped according to self-incompatibility categories. These categories were defined by an incompatibility index (SI = seed set after self pollination/seed set after cross- pollination) as follows: self-incompatible, SI = 0; slightly compatible, 0 < SI < 0.149; compatible, 0.15 < SI < 0.49; and fully compatible, SI > 0.5. Different letters indicate significant differences (Tukey-Krammer test, $P < 0.05$). Data gathered for *Flourensia cernua* in the Southwestern Chihuahuan Desert. This hand pollination experiment involved 50 plants from dense scrubs and 50 plants from diffuse scrubs.

Figure 3. Values of inbreeding depression (δ) associated with geminability (number of viable seeds/total number of seeds) in 45 partially self-incompatible individuals. Plants were grouped according to self-incompatibility categories. These categories were defined by an incompatibility index (SI = seed set after self pollination/seed set after cross- pollination); slightly compatible, 0 < SI < 0.149 open bars; compatible, 0.15 < SI < 0.49 dashed bars; and fully compatible, SI > 0.5 closed bars. Data gathered from *Flourensia cernua* populations in the Southwestern Chihuahuan Desert.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Percentage
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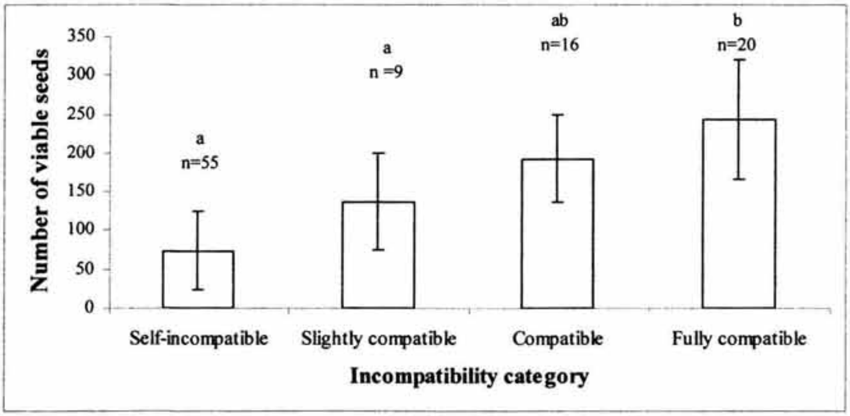
Self-incompatible
Slightly compatible
Compatible
Fully compatible
Incompatibility category


Number of viable seeds
350
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150
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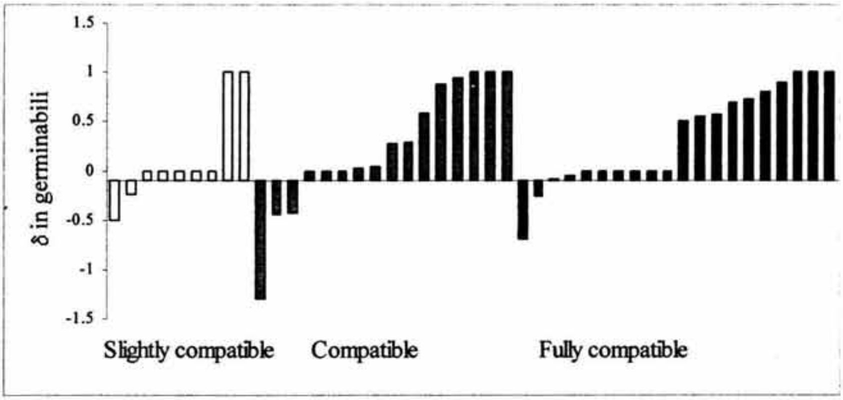
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Slightly compatible
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Genetic structure and outcrossing rates in *Flourensia cernua* (Asteraceae) growing at different densities in the South-western Chihuahuan Desert.

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Genetic structure and outcrossing rates in *F. cernua*

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ABSTRACT

- **Backgrounds and aims**: *Flourensia cernua* is a partially self-incompatible, wind-pollinated shrub that grows in two scrub types of contrasting densities. We expected the difference in plant density to affect the amount of genotype availability and thus to find higher outcrossing rates and less genetic differentiation in high density sites.
- **Methods**: At five high-density sites and at five low-density sites, 11 allozyme loci were analysed in adult. Outcrossing rates were estimated using 5 allozyme loci sampled from 8 families from each scrub type.
- **Key results**: High levels of genetic variation were found at all sites (ranging from $P = 82$ to 100%, $H_e = 0.33$ to 0.45, and $H_o = 0.41$ to 0.59). Heterozygotes were found in excess ($F_{IS} = -0.15 \pm 0.06$ s.d.) suggesting that natural selection favours heterozygosity, and there was little differentiation among sites ($F_{ST} = 0.08 \pm 0.02$ s. d.). Life history attributes such as long-lived habit and wide geographic distribution as well as by the presence of a self-incompatibility system may explain these results. Outcrossing rates did not differ from 1.0 in both scrub types, and there was no genetic differentiation between scrub types ($F_{ST} = -0.01 \pm 0.004$ s. d.).
- **Conclusions**: The high rate of outcrossing favoured by partially incompatibility may generate unrestricted gene flow among scrubs types and thus may explain the lack of differentiation between scrub types. High heterozygosity could be expected in long-lived plants of arid zones as they confront a variable and stressing environment.

Key words. Density, outcrossing rates, genetic structure, genetic variability, heterozygosity excess, self-incompatibility, *Flourensia cernua*

INTRODUCTION

The genetic structure (*i. e.,* the amount of genetic variability within and among populations) has an impact in ecological adaptation and evolution of plant species plants (Loveless and Hamrick, 1984; Booy *et al.,* 2000). For instance, it has been stated that high genetic variability at the population level is important in situations of stress and also favorable when individuals grows in heterogeneous environments (Hedrick, *et al.*, 1976; Ewing, 1979; Gillespie and Turelli, 1989). This kind of reasoning made Nevo and Broyles (1988) propose that organisms inhabiting arid and semiarid zones should display higher levels of genetic variability at the population level than those inhabiting other habitats. On the other hand, demographic factors such as density and population size influence the genetic structure of plant species (Loveless and Hamrick, 1984; Levin, 1988; Brown and Schoen, 1992). Life history traits like mating system and seed dispersal mechanisms has also an influence in the genetic structure of plant species (Hamrick and Godt, 1990; Hamrick and Godt, 1996).

Variability in outcrossing rates as a function of plant density is one of the most important factors that determine genetic structure (Farris and Mitton, 1984; Levin, 1988; Eguiarte, Pérez-Nasser and Piñero, 1992). In particular, in anemophyllous species outcrossing rates in general are higher at higher plant densities (Farris and Mitton 1984; Vaquero *et al.,* 1988; Knowles *et al.*, 1987), and may be due to the proportionally smaller representation of own pollen with respect to alien pollen (Farris and Mitton, 1984). Vaquero *et al.*, (1988) also found that decreased outcrossing rates in less dense sites were partially due to a breakdown of the incompatibility system in rye.

The genetic incompatibility system is a pre-zygotic mechanism that by preventing selfing and biparental inbreeding assures strict outcrossing (Charlesworth and Charlesworth, 1987). The presence of this system is documented among 50% of all angiosperms (de Nettancourt, 2001).

The Asteraceae family presents a sporophytic self-incompatibility system, although a partial breakdown of the incompatibility system has been found in several species (Lane, 1996, de Nettancourt, 2001). This breakdown allows some plants to produce seeds through selfing, and lead to variation in outcrossing rates in *Centaurea solstitialis* (Sun and Ritland, 2000).

The presence of the genetic incompatibility system and entomophyllous syndrome of pollinization in Asteraceae are thougth to be ancestral traits (Stebbins, 1970; Lane, 1996). However in arid, windy and/or cold habitats, lack of pollinators could led to a shift of entomophylous pollinization syndrome to an anemophylous one (Stebbins, 1970; Levin, 2000). In *Espeletia* (an Andinean genus) this shift in pollination syndrome is related with a breakdown in the self incompatibility system (Berry and Calvo, 1989). In this sense Asteraceae species from arid zones are interesting systems to test hypothesis about effects of wind pollination and incompatibility systems and the effect of both in outcrossing rates and in genetic structure.

In addition to outcrossing rates and the general mating system, seed dispersion life history traits can also have a strong influence on the genetic structure of plant populations (Loveless and Hamrick, 1984; Hamrick and Godt, 1996; Booy *et al.,* 2000). For instance, it is well known that genetic differentiation can be very high if outcrossing rates are low and seeds are only locally dispersed, (Allard *et al.,* 1968; Brown and Schoen, 1992, Hamrick and Godt, 1996). On the other hand, even moderate rates of outcrossing may prevent population subdivision, especially if pollen and seeds can be transported for large distances (Schaal, 1980; Slatkin, 1985). Pollen and seed flow are highly leptokurtic, therefore a higher gene flow is expected when plants grow more clumped (Levin and Kester, 1974; Levin, 1988).

Flourensia cernua (Asteraceae: Heliantheae), a long-lived shrub characteristic of the Chihuahuan Desert (Mac Mahon, 1989), is distributed in the south-western portion of its range among two scrub types of contrasting densities: high-density scrubs (824 individuals / ha ± 187 s.

e.) and low-density scrubs (11 individuals / ha ± 2.4 s. e.; M. Ferrer *et al.*, unpublished data. It is a wind-pollinated species, with hermaphroditic protandrous flowers (the fisrt two days as male and three last days as female). The species presents a self-incompatibility system, although some individuals show a breakdown of the system and are self-compatible at different levels (M. Ferrer *et al.*, chapter two). The production of viable seeds after selfing is low (2.4% of flowers produced a viable seed); but after cross pollination treatments the production increases to 20% of pollinated flowers (M. Ferrer, *et al.*, chapter two). In this *sense F. cernua* is a pseudo self-fertilizated species (*sensu* Levin, 1996), that is, some individuals can produce fruits after self-fertilization, but offspring derived from cross-pollination have a larger fitness than those derived from self-pollination. The seeds are primarily dispersed by gravity and subsequently by water flows; germination rates are low and recruitment is sporadic (Mauchamp *et al.* 1993).

In this study, we evaluated the effect of population density on genetic variation, genetic structure, and outcrossing rates in this shrub. We predicted that this species would follow the patterns found in most wind pollinated species: in less dense sites a decreased outcrossing rate will be found due either to a lower proportion of exogamous pollen or to a local breakdown of the incompatibility system. As a consequence, we also expected that genetic diversity would be lower in low-density sites, thus fewer genotypes and alleles would be found in the low-density scrub, and this will be expressed as genetic differentiation between scrub types.

MATERIALS AND METHODS

Study species

This study was done on the Mapimí Biosphere Reserve, Durango, Mexico (26° 40' N and 103° 40' W; 1,100 m above sea level; 264 mm of precipitation per year, 72% of which falls between June and September, Montaña, López-Portillo and Mauchamp, 1990). High-density scrubs are found on lower bajadas, sloping less than 1.5%; whereas low-density scrubs occur on upper bajadas sloping more than 3% (Montaña, 1990). These two types of scrub do not differ in general climatic or edaphic characteristics, except for a slightly higher clay content in dense scrub soils and higher water availability due to runoff rainwater redistribution (Cornet *et al.*, 1992, Galle *et al.*, 2001). In the high-density scrubs, *F. cernua* is the dominant shrub, along with *Hilaria mutica* and *Prosopis glandulosa* var. *torreyana* (Montaña, *et al.,* 1990). In the low-density scrubs the dominant species are *Opuntia rastrera* and *Larrea tridentata* (Montaña, 1990).

Sampling

Sampling was carried out at five high-density sites and at five low-density sites. Sites were randomly selected within an area of 20 km^2 (7 km^2 of high-density scrubs and 13 km^2 of low-density scrubs). Distance between the centres of the sites ranged from 0.41 km to 5.74 km, with an average of 3 km (Figure 1). Sites were divided into 10 m X 10 m quadrants. Within each quadrant, a single adult individual was chosen in a central position. Foliar tissue was collected from these individuals in October 1998 for genetic analyses. Following this scheme, foliar tissue was collected from 120 individuals found in high-density scrubs and 125 individuals occurring in low-density scrubs (with an average of 25 individuals per site). Samples were transported to the laboratory in liquid nitrogen where frozen tissues were maintained and stored in an ultra-cold freezer (REVCO) at -70^0 C.

To estimate outcrossing rates, fifteen individuals were selected at random from the high-density scrubs and fifteen from the low-density scrubs. In February 1999, foliar tissue as well as all the seeds produced by each of these individuals were collected and treated as 30 different genetic families in electrophoresis analyses from mother tissue and from seedling tissue. These seeds were taken to the laboratory in paper bags and maintained at room temperature while foliar tissue was transported to the laboratory in liquid nitrogen where frozen tissues were maintained and stored in an ultra-cold freezer (REVCO) at -70^0 C. During the months of July and August of 1999, all seeds were allowed to germinate in environmental chambers on a substrate of moistened cotton. We obtained seedlings only for eight out of the fifteen families from each scrub type due to a low viability of the seeds (see Valencia-Díaz and Montaña, 2003). Seedlings were maintained under a photoperiod of 12 hours light (at 26 ° C), 12 hours darkness (at 16 ° C), and 60% humidity until harvesting at three weeks of age.

Electrophoresis technique

Leaves from adults were macerated with liquid nitrogen and then 75 mg of the tissue was mixed with 375 µl of extraction buffer. Seedlings were macerated with 100 ml of extraction buffer. The buffer consisted of one part Veg II (Cheliak and Pitel, 1984) and three parts YO solution (Yeh and O'Malley, 1980). The suspension was centrifuged at 1,000 rpm for three minutes at 4° C. The supernatant was absorbed using filter paper wicks (25 mm wide x 15 mm long; Whitman, number 17) and then the wicks were stored in an ultra-cold freezer (REVCO) at -70°C until needed.

Allozyme analysis was undertaken on horizontal starch gels (11%; Sigma Chemical Co. St. Luis, MO). Used buffer systems were Poulik pH 8.0 and pH 7.6 (gel and electrode, respectively; modified from Piñero and Eguiarte, 1988), and L-Histidine pH 6.3 and pH 7.0 (gel and electrode, respectively; also modified from Piñero and Eguiarte, 1988). The Poulik gel was allowed to run at 270 V for 12 minutes, after which time the wicks were removed, and the gels allowed to continue running until the front had reached eight cm (after approximately 6 hours). The L-Histidine gels ran at 30 V for 15 minutes, after which time the wicks were removed and the gels allowed to run for 12 hours until the front attained 10 cm. In the Poulik system, the following enzymes were stained: Menadion reductase (Mnr, E. C. [Enzyme Comission number] 1.6.99), Leucyl aminopeptidase (Lap, E. C. 3. 4. 11. 1), Isocitrate dehydrogenase (Idh, E. C. 1. 1. 4. 2), Phosphoglucose isomerase (Pgi, E. C. 5. 3. 1. 9), Superoxide dismutase (Sod, E. C 3. 1. 3. 2), Acid phosphatase (Acph, E. C. 3. 1. 3. 2), and Esterase locus 1 (Est, E. C. 3. 1. 1. 1). In the L-Histidine system, the Est (locus 2), Peptidase (Pep, E. C. 3. 4. 11) and Peroxidase (Apx, E. C. 1. 11. 1. 7) enzymes were stained. All 11 enzymes staining protocols were modified from Soltis *et al.* (1983), Vallejos (1983) and Piñero and Eguiarte (1988). In adults, all enzymes showed readable banding patterns. However, in seedlings a consistently interpretable band was only achieved with the Mnr, Acph, Apx (loci 1 and 2) and Est (locus 1) enzymes. The loci and alleles that migrated most rapidly were designated as 1.

Genetic variability

For the adults in each site, estimates were made of the proportion of polymorphic loci (P, 95% criterion), the expected (H_e) and observed (H_o) levels of heterozygosity, and the average fixation index (F) (Hedrick, 2000). In addition, chi-square tests were used to analyse deviations of observed genotypic frequencies from expected Hardy-Weinberg equilibrium frequencies (Sokal and Rohlf, 1995), as well as to analyse the heterogeneity of allelic frequencies among populations (Workman and Niswander, 1970). To determine if there were differences between high and low-density scrubs in these parameters of genetic variability in adults, data were submitted to a Kruskall-Wallis non-parametric ANOVA (Sokal and Rholf, 1995). For this test, sites were considered to be repetitions within each of the two scrub types.

Genetic structure

To evaluate the genetic differentiation among adult plants, between high-density and low-density scrub types, Wright's F statistics (Wright, 1965) were estimated according to the method described by Weir and Cockerham (1984). The TFPGA program (Miller, 1996) used to undertake this analysis considered the two scrub types as populations and the individual sites as subpopulations. Standard deviations and 95% confidence intervals for mean F statistics were obtained through a Jackknife test. The χ^2 test of Li and Horvitz (1953) was used to test if F_{IS} and F_{IT} values per locus differ from 0, and the χ^2 test of Workman and Niswander (1970) was used to test if F_{ST} values per locus were different from 0. Additionally, cluster phenograms were obtained and graphically presented using Nei's genetic distances (1978) between subpopulations and UPGMA algorithms (Sokal and Michener, 1958). Finally, the correlations between estimates of Nm (Slatkin, 1993) and values of $F_{ST}/1-F_{ST}$ (Rousset, 1997) with the geographic distances between sites was also calculated, and statistical significance analysed by a Mantel test after 1,000 permutations (Mantel, 1967).

Outcrossing rates

Outcrossing rates were estimated for each of the 16 families and also for each scrub type considering the eight families from each scrub type. Electrophoretic data from mother tissue and from seedling tissue were used for this purpose. A range of eight to fifteen seedlings was obtained per mother providing a total of 92 individuals from the high-density scrubs and 100 individuals from the low-density scrubs. The mean outcrossing rates per scrub type for single and multiple loci (t_s and t_m, respectively) were estimated using the mixed mating model proposed by Ritland and Jain (1981) and the Multilocus Mating System Program (MLT; Ritland, 1990).

Ninety-five percent confidence intervals were calculated as the region comprised between the 25th and 975th value of 1,000 bootstrapped values of t_s and t_m mean values (previously sorted by their values), obtained by resampling within the families.

RESULTS

Genetic variability

The levels of genetic diversity of adult *Flourensia cernua* at each site were estimated as the proportion of polymorphic loci (P), and expected and observed heterozygosity (H_e and H_o respectively Table 1). These estimates indicate high levels of genetic variation within each site, but no differences between the two contrasting plant densities (Kruskall-Wallis ANOVA, $P > 0.05$, Table 1). Estimates of P ranged from 81.82 to 100%, while H_e varied from 0.33 to 0.45 and H_o ranged from 0.41 to 0.59.

The mean value of the fixation index per loci per scrub, F, for all loci was negative, indicating that there was an excess of heterozygotes among adults ($N = 145$ individuals, 11 loci; Table 1). Observed levels of heterozygosity in five loci from the high-density scrub sites and in four loci from the low-density scrub sites were larger than those expected by the Hardy-Weinberg model ($P < 0.05$), varying from one locus to four loci between sites (Table 1).

Genetic structure

Estimates of F_{IS} for adults indicate a general excess of heterozygotes as they were significantly negative for most (six) of the loci (Acph, Est1, Est2, Idh, Mnr and Pep), while only significantly positive for two loci (Apx1 and Apx2, Table 2). Mean F_{IS} was negative and differed significantly from zero ($F_{IS} = -0.1463$, -0.2530 to –0.0276, 95% confidence interval; Table 2).

Estimates of F_{IT} in adults were also negative and significantly different from zero (Mean F_{IT} = -0.2475, -0.3270 to –0.1412, 95% confidence interval; Table 2). Nine out of eleven loci showed a significant excess of heterozygotes (Acph, Est1, Est2, Idh, Lap, Mnr, Pep, Pgi and Sod1), whereas none of the loci showed a significant excess of homozygotes (Table 2).

Estimates of F_{ST} among sites were statistically different from zero (F_{ST} = 0.0807, $P < 0.05$; Table 2), indicating that despite the fact that the genetic differences are low, there is significant differentiation among the 10 sites. Seven estimates of F_{ST} among the 10 sites were significantly different from zero in the loci Acph, Apx1, Apx2, Est1, Est2, Lap, Pgi and Sod1 (Table 2). On the other hand, if we only compare the two scrub types, there is no genetic differentiation (F_{ST} = -0.0130, $P > 0.05$; Table 2), negative values of F_{ST} are usually considered an statistic artefact and is not different from zero.

As suggested by the low F_{ST} values among sites values, the Nei's genetic distances (*D*) for adults estimated between paired sites were relatively small (*D* = 0.0037; range –0.022 to 0.1366). Average *Nm* was 3.47 ± 1.47 s.d. for all pairs of sites, in sites within low density scrubs the *Nm* was 3.27 ± 2.48 s. d. and in sites within high density *Nm* was 2.48 ± 0.87 s. d. (Table 3). Pairs estimates of *Nm* (M) and geographic distances correlation was -0.133, but did not differ significantly from zero (Mantel test, original Z = 747.30, mean *Z* after permutations = 773. 91, *P* = 0.55 and 0.45 upper and low tail probability respectively ,see table 3), the correlation analysis using Rousset estimates did not improve the correlation (*r* =-0.133, Mantel test, original Z = 14.56, mean *Z* after permutations = 15.08, *P* = 0.80 and 0.19 upper and low tail probability respectively. Both results indicate no isolation by distance. High and low density scrubs intermingle in the phenogram, suggesting that genetic similarity depends more on geographic distribution than on the scrub type identity (Figs. 1 and 2).

Outcrossing rates

Mean estimates of t_s (single-locus outcrossing rate) and t_m (multi-locus outcrossing rates) were high in both types of scrub (1.19 ± 0.19 s. d. and 1.19 ± 0.21 s. d. for the high density scrubs and 0.83 ± 0.27 and 0.79 ± 0.25 s. d. for the low-density scrubs, respectively). The t_s was slightly higher than t_m in both scrub types, however this difference was not significant. The 95% confidence intervals for t_s and t_m comprised a large region, suggesting that progeny may have been derived both from selfing and outcrossing in both scrub types (95% confidence intervals ranging 0.66 to 1.49 and from 0.23 to 1.37 for t_s and from 0.67 to 1.47 and from 0.23 to 1.22 for t_m for high density and low density scrubs, respectively). Notwithstanding, no differences in t_m were detected between the two types of scrub, given that confidence intervals for both types overlapped and neither one differed significantly from 1.00.

DISCUSSION

We studied 10 sites of *F. cernua*, and we found high levels of genetic variation, significant genetic differentiation and an excess of heterozygotes. Outcrossing rates are high, and significantly not different from one, but contrary to our expectations, we found neither effects of the plant density on levels of genetic variation, genetic structure or outcrossing rates, nor signals of isolation by distance. These results may be explained by life history attributes such as woody long-lived habit and wide geographic distribution as well as by the presence of a self-incompatibility system. We suggest that this pattern is enhanced by natural selection favouring heterozygosity, and that high gene flow between scrub types prevents genetic differentiation.

Variability and genetic structure

Genetic variability in *Flourensia cernua* is relatively high , while genetic differentiation among sites is low but significant. On the other hand, there are no genetic differences between the two scrub types (*i. e.* high-density scrub and low-density scrub). Similar patterns (genetic variability within populations and not among them) have been reported in several long-lived, widespread and outcrossing species (see review in Hamrick, *et al.,* 1992, Hamrick and Godt, 1996). Actually, our estimates of genetic variability within sites were higher than the averages for long-lived, widespread and outcrossing species of several families, and for 101 Asteraceae species ($P = 45.3$, $H_e = 0.127$; Hamrick and Godt 1996) and also greater than the values reported for other woody or succulent species occurring in arid zones (Martínez-Palacios, *et al.,* 1999 and cites therein). Genetic variability in *F. cernua* is comparable to that encountered in *Prosopis glandulosa* var. *torreyana,* a long-lived and preferentially cross-pollinated species common to the Chihuahuan Desert ($H_o = 0.45 \pm 0.03$ s. d., $F = - 0.46 \pm 0.1$ s. d., $t_m=1$, Golubov *et al.,* 1999).

Our results clearly indicate a significant excess of heterozygotes in the adults for 6 out of 11 loci for F_{IS} and in 9 out of 11 loci for F_{IT}. A significant excess of heterozygotes among adults may be explained in general by natural selection favouring heterozygosity (Hedrick, 2000) and perhaps for some loci by a linkage between the allozyme loci and the loci that control self-incompatibility system (Leach, 1988). In the first case, the excess of heterozygosity comprises various loci and increases along the life cycle development (*i. e.* Eguiarte, *et al.,* 1992; Alvarez-Buylla and Garay, 1994; González-Astorga et al, 2003) while in the second case, only one or two allozyme loci shows this significant excess (*i. e.,* Manganaris and Alton, 1987; O'Leary and Boyle, 1998).

In *F. cernua*, seed viability in general is low (ca. 9%), but the viability of progeny derived from outcrossing is higher than that of offspring derived from selfing (M. Ferrer, *et al.*, chapter two). These results suggest that few self-sired seedlings could survive until adult stage due to inbreeding depression. Therefore, adult populations are expected to be composed mainly of outcrossed and heterozygous plants. The high level of heterozygosity in *F. cernua* may confer certain selective advantages. For example, heterozygosity plays an important role in species that inhabit stressful environments (Rainey, *et al.*, 1987; Mopper *et al.*, 1991). Plants in arid zones face great temporal environmental heterogeneity that is related primarily to the unpredictability of precipitation. For this reason, high genetic diversity might be favoured (Nevo and Beiles, 1989). However, we found no differences between the two types of contrasting densities, and the performance of heterozygote individuals is not known for life history stages other than germination in this species. For these reasons, selection favouring heterozygosity must remain a hypothesis yet to be tested.

On the other hand, 1 to 4 loci per site had a significant excess of heterozygotes and in these cases only the Mnr locus had always a significant heterozygote excess in all sites, suggesting that this locus may be linked with the incompatibility locus (see Appendix 1). After founding a significant heterozygote excess, O'Leary and Boyle (1998) demonstrated that the Lap-1 locus is linked with self incompatibility locus in the Christmas cactus *Schlumbergera*. More genetic studies are needed to understand linkage among the Mnr locus and self-incompatibility locus in *F. cernua*.

Genetic structure

Pollen and seed movement may influence the low genetic differentiation between populations of *F. cernua*. The pollen of this species is dispersed by wind (Mauchamp, *et al.*, 1993). Although direct estimates of pollen movement are lacking in this study, other studies of anemophyllous species have found that pollen is capable of being dispersed for distances greater than 3 km (Ratchke and Lacey, 1985; Levin, 1988). Geographic distances between sites in this study ranged from 0.5 km to 6 km (Fig. 1), and for this reason, it is doubtful that the physical distances between sites could be a barrier to pollen dispersal.

The seeds of *F. cernua* disperse primarily by gravity and secondarily by surface water runoff after rare heavy rains (Montaña *et al.,* 1990; Mauchamp *et al.,* 1993). Surface water runoff could easily allow the movement of seeds from low-density to high-density scrub associations, since the latter are always located down slope. Individuals in low-density scrubs are typically distributed around ephemeral streams (*i. e.*, arroyos), which run from the upper bajadas down to the lower bajadas where high-density *F. cernua* scrubs are found (Fig. 1). The surface water runoff might have caused a directional flow of seeds (*i. e.*, down slope) at these sites, thus may explain the pattern of genetic similarity observed in this study (Figs. 1 and 2).

Effect of density on outcrossing rates

We found no significant differences among ts and tm estimates, and no deviations from the expectation of the mixed mating model. However, constraints of the mixed mating model could be observed if we considered that partial negative assortative mating is occurring in F. cernua due to the presence of the self- incompatibility system. Because departures from the mixed mating model affect less the multilocus outcrossing rates, tm can be regarded as the more reliable estimate (Ritland and Jain, 1981, Ritland, 1990, Eguiarte et al., 1992). The outcrossing rates in F. cernua are high and not different from one, but also the estimated variances are very large. We suggest that this variance may be due to the fact that, as controlled self-pollinations revealed, most plants are completely self-incompatible, but some of them display a variable degree of self-compatibility, that can be very high in some cases (M. Ferrer *et al.* chapter two).

Contrary to our initial expectations, population density did not affect outcrossing rates in *F. cernua*. The results of this study agree with results for some other species, including *Picea abies* (a wind pollinated conifer, Morgante, *et al.*, 1991), *Solidago sempervivens* (an incompatible Asteraceae, Innes and Hermanutz, 1988), and *Cakile maritima* (an incompatible Brassicaceae, Thrall *et al.*, 2000). Long distance wind dispersion of pollen and the presence of a partial self-incompatibility system in *F. cernua* may be the principal factors determining the lack of statistical association between population densities and outcrossing. Morgante *et al.* (1991) associated the lack of correlation between outcrossing rates and plant density in *P. abies* with high viability and dispersion capacity of pollen (dispersion greater than 5 km). The presence of a self incompatibility system is the reason for the absence of a density effect on outcrossing rates in *Solidago sempervivens* (Innes and Hermanutz,1988) and in *Cakile maritima* (Thrall *et al.*, 2000).

High heterozygosity could be favourable in long-lived plants of arid zones as they confront a stressing environment, thus high outcrossing rates should be favoured despite a high spatial variability in population density. We suggest that high outcrossing rates in long lived desert plants would result in high gene flow, and little genetic differentiation as it is known in conifers, as opposed to self compatible, bee pollinated species living in spatially restricted ranges.

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Table 1. Genetic variability in F. cernua adults. Data from high density scrubs (five sites, 120 individuals) and low density scrubs (five sites, 125 individuals). Number of individuals (N), proportion of polymorphic loci 95% criterion (P), expected heterozygosity (He), observed heterozygosity (Ho) and fixation index (F). The four last columns indicate number of loci where statistical test show departures from Hardy-Weinberg equilibrium (Test), number of loci with significant excess of heterozygosity (Excess), significant deficiency of heterozygosity (Deficiency) and number of fixed loci (Fixed). Data estimated for 11 loci in 9 enzymes. χ2 values for Kruskal-Wallis ANOVA test among scrub types are shown in the last row.

	N	P	He	Ho	F	Test	Excess	Deficiency	Fixed
High density									
A	18.55	100.00	0.42	0.46	-0.11	11	1	0	0
B	23.09	90.91	0.36	0.50	-0.41	10	4	1	1
C	18.36	100.00	0.41	0.46	-0.11	11	2	1	0
D	18.50	90.00	0.38	0.50	-0.32	9	2	0	1
E	9.91	81.82	0.38	0.57	-0.50	9	3	0	2
Mean	17.68	92.55	0.39	0.50	-0.29				
s. e.	2.14	3.43	0.01	0.02	0.08				
Low density									
F	18.50	100.00	0.37	0.41	-0.12	11	2	1	0
G	18.09	100.00	0.39	0.53	-0.37	11	3	0	0
H	23.18	100.00	0.45	0.59	-0.30	11	3	0	0
I	15.40	100.00	0.41	0.50	-0.21	10	1	0	0
J	18.36	90.91	0.33	0.41	-0.25	11	1	1	0
Mean	18.71	98.18	0.39	0.49	-0.25				
s. e.	1.25	1.82	0.02	0.03	0.04				
χ^2		1.67	0.40	0.40	0.40				

Table 2. Wright's F statistics for *F. cernua* adults. Estimates of F_{ST} are presented per sites (sites) and scrub types (scrub). Significant differences from zero are denoted per loci and in average (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; $N = 125$ and 120 individuals in low and high density scrubs respectively, 11 loci in 9 enzymes). 95% confidence intervals and standard deviations were obtained through a Jacknife test.

	F_{IS}		F_{ST}		F_{ST}	F_{IT}	
			Sites		Scrub		
Acph	-0.4205	***	0.0158		0.0049	-0.4432	***
Apx1	0.1651	*	0.2138	***	-0.0365	-0.062	
Apx2	0.2031	**	0.0815	***	-0.001	0.1324	
Est1	-0.1773	**	0.0672	***	-0.0203	-0.2621	***
Est2	-0.221	**	0.1055	***	-0.0279	-0.365	***
Idh	-0.262	**	0.0347		-0.009	-0.3074	***
Lap	-0.0559		0.1030	***	0.004	-0.1771	*
Mnr	-0.3239	***	0.0338		-0.0126	-0.3702	***
Pep	-0.22	**	0.0392		-0.0095	-0.2698	***
Pgi	-0.0811		0.1297	***	-0.0148	-0.2422	***
Sod1	-0.1439		0.0984	***	-0.0278	-0.2688	***
Mean	-0.1463		0.0807*		-0.013	-0.2475	
s. d.	-0.0624		0.0187		0.0044	0.0503	
Upper limit	-0.0276		0.1197		-0.0058	-0.1412	
Lower limit	-0.253		0.0504		-0.0216	-0.327	

Table 3. Gene flow estimates (Slatkin, 1993; above diagonal) and geographic distances (under diagonal, in km) among sites pairs in *F. cernua* adults. A, B, C, D and E are the 5 sites in high density scrubs, while *F, G, H, I* and *J* are the 5 sites in low density scrubs.

	A	B	C	D	E	*F*	*G*	*H*	*I*	*J*
A	*****	1.972	6.210	7.563	10.167	4.337	10.526	75.508	6.079	3.400
B	1.01	*****	1.418	1.352	1.348	1.400	1.723	1.508	2.063	1.398
C	5.74	4.73	*****	1.726	5.591	3.039	4.121	3.227	1.570	4.503
D	5.36	4.24	0.56	*****	1.847	1.235	2.939	5.306	4.183	1.413
E	0.41	0.49	5.06	5.14	*****	6.470	3.470	9.256	1.962	5.002
F	3.45	3.41	6.15	5.03	3.60	*****	4.002	2.963	1.319	3.119
G	2.93	2.63	5.66	5.21	3.04	1.50	*****	3.321	2.029	2.194
H	2.85	2.44	5.21	4.84	2.81	1.95	1.58	*****	4.760	2.291
I	4.58	3.90	2.66	2.59	4.28	3.86	3.56	2.85	*****	1.147
J	3.00	2.48	3.71	3.34	2.81	3.04	2.44	2.10	1.95	*****

Figure 1. Distribution of *Flourensia cernua* sites. High density scrubs (A, B, C, D and E) and low density scrubs (*F, G, H, I* and *J*) in the South-western Chihuahuan Desert. Aerial photography (original scale 1:75,000, INEGI, 1986).

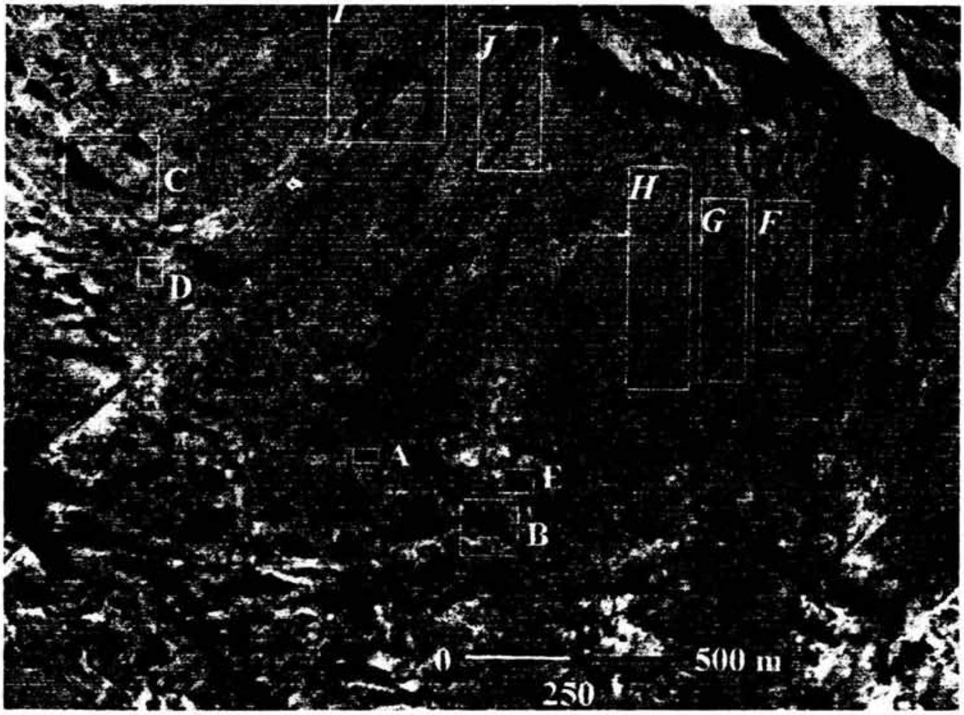
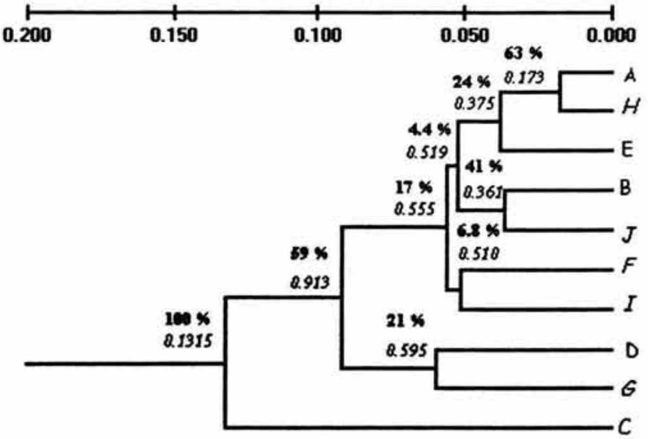


Figure 2. Cluster phenogram of *Flourensia cernua* populations in the South-western Chihuahuan Desert. High density scrubs (*A, B, C, D* and *E*) and low density scrubs (*F, G, H, I* and *J*). The dendrogram was constructed using Nei's genetic distances (Nei, 1978) and UPGMA algorithms (Sokal and Micherner, 1958). UPGMA Cluster using Nei's (1978) original distance for each node (italic text); percentage of similar replicates, results from bootstrapping (1000 permutations bold text).



APPENDIX 1

Allelic frequencies of *F. cernua* populations in high density scrubs (High) and low density scrubs (Low). For locus with two alleles, allelic frequencies for just one of the two alleles are showed, similarly for locus with three alleles, allelic frequencies for two alleles are showed.

High	1	A	B	C	D	E
	Allele	Freq.	Freq.	Freq.	Freq.	Freq.
LAP	1	0.3947	0.8750	0.4130	0.4091	0.3000
MNR	1	0.5000	0.4500	0.3125	0.6250	0.5000
PGI	1	0.0750	0.0278	0.0000	0.0000	0.0000
	2	0.8500	0.6667	1.0000	1.0000	1.0000
IDH	1	0.4167	0.3158	0.5800	0.3750	0.4000
EST1	1	0.0750	0.3333	0.1458	0.0750	0.4500
SOD	1	0.4667	0.5417	0.5000	0	0.4500
ACPH	1	0.5000	0.0250	0.3750	0.4688	0.5000
PEP	1	0.4737	0.3684	0.3043	0.6000	0.3889
APX1	1	0.5278	0.4250	0.7600	0.3500	0.7500
APX2	1	0.3421	0.4211	0.1200	0.1579	0.3000
EST2	1	0.1111	0.0294	0.0000	0.4250	0.0000
	2	0.8333	0.6176	0.8182	0.5250	1.0000
Low		F	G	H	I	J
	Allele	Freq.	Freq.	Freq.	Freq.	Freq.
LAP	1	0.4167	0.4643	0.3000	0.3333	0.4688
MNR	1	0.4250	0.6500	0.5000	0.5000	0.3000
PGI	1	0.0625	0.0000	0.1667	0.5000	0.0000
	2	0.9063	0.9500	0.7917	0.2917	1.0000
IDH	1	0.4688	0.4706	0.4773	0.3611	0.1750
EST1	1	0.2368	0.0250	0.3000	0.1944	0.2500
SOD	0	0	0.2667	0.5000	0	0.4231
ACPH	1	0.3000	0.4333	0.6000	0.2273	0.2941
PEP	1	0.5294	0.3158	0.6250	0.3182	0.5278
APX1	1	0.9500	0.7500	0.4800	0.1944	0.8750
APX2	1	0.5750	0.3750	0.3478	0.3611	0.1000
EST2	1	0.0263	0.1316	0.1458	0.2917	0.0000
	2	0.8947	0.6053	0.7708	0.7083	0.8889

DISCUSIÓN GENERAL Y CONCLUSIONES

En esta discusión integraremos los resultados de todos los capítulos para subrayar los diferentes factores que limitan la adecuación de *Flourensia cernua* y discutiremos la importancia de la variación del sistema reproductivo (*i. e.* sistema de auto-incompatibilidad genética y tasas de entrecruzamiento) y la diversidad y estructura genética y su relación con la densidad poblacional.

Limitación de la adecuación de F. cernua

La reproducción sexual de una planta se puede esquematizar como una cadena de eventos fenológicos y demográficos que incluyen la producción de flores, la polinización de las mismas, la producción de frutos, producción de semillas, la germinación, el establecimiento de plántulas, y el crecimiento y supervivencia de las mismas hasta alcanzar el estadio de adultos (ver diagrama 1). Todos estos eventos son componentes de la adecuación de un genotipo x (w_x); la producción de flores y semillas viables constituyen la fecundidad de un genotipo (m_x) y el establecimiento y supervivencia de los individuos pueden resumirse en el componente de supervivencia del genotipo (l_x).

Las especies de larga vida que habitan en zonas áridas se caracterizan por tener una gran fecundidad, bajo establecimiento de plántulas y alta mortalidad de plántulas y juveniles (Shreve, 1929; Westoby y Watson, 1997). Sin embargo, en *F. cernua* encontramos que la adecuación de los individuos de no sólo está limitada por un bajo reclutamiento y una alta mortalidad de plántulas (*i. e.* en el componente de supervivencia), sino que existe una fuerte limitación en el componente de fecundidad (capítulo 1).

Este patrón puede esquematizarse en un diagrama que muestra las diferentes limitaciones tanto en la fecundidad como en la supervivencia de *F. cernua* (ver diagrama 2). En este diagrama se presentan los patrones detectados en los diferentes trabajos que integran la tesis (lado izquierdo diagrama 2) y los procesos que están involucrados en la limitación de la reproducción sexual de la especie (lado derecho diagrama 2).

Como se discutio en el capítulo 1 de la tesis, los procesos que limitan fuertemente la reproducción sexual de la especie son: una aborción de frutos alta y una germinabilidad de las semillas baja. Existe también una limitación en la reproducción sexual de la especie asociada con la depredación pre-dispersión de los frutos y con la baja disponibilidad de polen compatible (capítulo 1). De todos estos factores limitantes el único que varía con el tipo de matorral es la depredación pre-dispersión, en este sentido es el único factor que podría estar afectado por la densidad poblacional. Para elucidar si la densidad poblacional afecta la depredación o si el depredador tiene una preferencia por el hábitat se necesita un nuevo diseño que incluya la manipulación de las densidades poblacionales en ambos tipos de matorral.

Sistema reproductivo de F. cernua.

La presencia del sistema de auto-incompatibilidad limita la adecuación de *F. cernua* al disminuir la cantidad de semillas que se producen (capítulo 2). El sistema de auto-incompatibilidad es un factor que restringe de manera importante el sistema reproductivo de la especie y este efecto sobrepasa de la densidad poblacional. En este sentido, podemos explicar en parte, la falta de diferencias entre los matorrales densos y difusos.

La limitación por polen es muy común en especies auto-incompatibles (Larson y Barret, 2000). En *F. cernua* la limitación por polen se manifiesta en dos sentidos: la cantidad de polen que llega a los estigmas y la calidad del polen (compatible o incompatible, capítulo 2). En el primer caso (cantidad de polen), la densidad poblacional afecta directamente la disponibilidad de polen, en los matorrales difusos hay una limitación mayor por la cantidad de polen (capítulo 2) y las plantas que crecen en grupos aislados tienen una menor producción de frutos. La disponibilidad de polen compatible afecta la fecundidad en los matorrales densos, como en los difusos (capítulo 2). La baja disponibilidad de polen compatible puede estar asociada a una diversidad alélica baja en el locus que controla la auto-incompatibilidad y/o a un agrupamiento espacial o temporal de individuos reproductivos que comparten el mismo fenotipo para el gen de la auto-incompatibilidad.

La disminución de alelos S conlleva a una disminución en la frecuencia de apareamientos compatibles por lo que la producción de semillas disminuye (De Mauro, 1993; Reinartz y Les, 1994). De manera similar, el agrupamiento de individuos con fenotipos similares para la auto-incompatibilidad disminuye la frecuencia de apareamientos compatibles y limita la producción de semillas (Brennan *et al.*, 2003). En ambos casos un rompimiento parcial en el sistema de auto-incompatibilidad garantiza que las plantas se reproduzcan (Levin, 1996).

Para conocer si la baja disponibilidad de polen compatible y/o un agrupamiento de parientes están asociados con el rompimiento del sistema de auto-incompatibilidad se requieren nuevas investigaciones que comprendan trabajos de polinización (cruzas recíprocas), con el fin de estimar el número de alelos S en las poblaciones, y estudios que describan la estructura genética a una escala más fina (p. ej. análisis de auto-correlación espacial) con marcadores más variables como los microsatélites.

El rompimiento parcial en el sistema de auto-incompatibilidad, permite la existencia de un sistema de apareamiento mixto en *F. cernua*, como lo indican las estimaciones de las tasas de entrecruzamiento (capítulo 3) y la auto-compatibilidad de cerca de un 45% de la población (capítulo 2). Los sistemas de apareamiento mixto son raros en especies anemófilas (Vogler y Kalisz, 2001), sin embargo, se ha reportado que en el género *Espeletia* el rompimiento del sistema de auto-incompatibilidad está asociado con el síndrome de polinización anemófila (Berry y Calvo, 1989). El género *Flourensia* es interesante para probar si el patrón observado en *Espeletia* es consistente, ya que ambos géneros tienen tanto especies polinizadas por viento como por animales y al pertenecer a la misma familia comparten el mismo sistema de auto-incompatibilidad (*i. e.* esporofítico). Un estudio que integre la valoración del sistema de auto-incompatibilidad en las especies del género *Flourensia* y la presencia de diferentes síndromes de polinización podría ayudarnos a compreder los patrones macro-evolutivos que dieron origen al rompimiento en el sistema de auto-incompatibilidad.

La evolución del sistema de apareamiento en *F. cernua* dependerá en gran medida del efecto de la depresión endogámica. La depresión endogámica es una de las fuerzas que regulan la evolución de los sistemas reproductivos (Lande y Schemske, 1985; Charlesworth y Charlesworth, 1987). En los modelos teóricos más restrictivos, el cambio de un sistema de polinización cruzada predominante a uno de auto-polinización, requiere que el efecto de la depresión endogámica sea muy bajo (*e. g.* δ menor a 0.5; Lande y Schemske, 1985; Charlesworth *et al.*, 1990). En las poblaciones de *F. cernua* la variación en δ es muy grande, algunos individuos tienen una $\delta < 0.5$ y algunos presentan $\delta > 0.5$.

Tanto la variación en los índices de depresión endogámica, como en el grado de auto-incompatibilidad pueden tener un efecto en el futuro del mantenimiento del sistema de entrecruzamiento de las poblaciones. Suponiendo que en las poblaciones sólo los individuos auto-compatibles estén dejando descendencia, se pueden plantear los siguientes escenarios: 1) Cuando $\delta < 0.5$, la frecuencia de individuos auto-compatibles en las poblaciones se incrementa, y se espera un incremento en las tasas de auto-polinización. 2) Cuando la $\delta > 0.5$, la frecuencia de individuos auto-compatibles en las poblaciones disminuye y se espera un incremento en las tasas de polinización cruzada. En *F. cernua* ambos casos se observan, y la auto-polinización como sistema de apareamiento sólo podría observarse en el caso de que la frecuencia de individuos con $\delta < 0.5$ sea mayor que la frecuencia de individuos con $\delta > 0.5$. En nuestro sitio de estudio no existen diferencias entre las frecuencias de individuos con $\delta < 0.5$ y $\delta > 0.5$, por lo que no podemos hacer predicciones sobre la evolución de esta especie de un sistema auto-incompatible a uno auto-compatible.

Sin embargo, creemos que los individuos auto-compatibles pueden mantenerse en la población porque tienen una mayor adecuación en comparación con aquellos que son parcialmente auto-compatibles o los que son auto-incompatibles (capítulo 2). Adicionalmente, en algunos casos la progenie derivada de auto-fertilización tiene una adecuación mayor o igual a la que tiene la progenie derivada de polinización cruzada, es decir el efecto de la depresión endogámica es bajo (capítulo 2).

Diversidad y estructura genética

Las predicciones sobre el efecto de la densidad en la diversidad y estructura genética de las poblaciones de la Reserva de la Biosfera de Mapimí, fueron hechas en un principio basándonos en la literatura de plantas anemófilas (capítulo 3). Sin embargo, nuestros resultados no concuerdan con estos patrones (*i. e.* tasas de polinización cruzada mayores en sitios densos, diversidad genética baja en poblaciones difusas y diversidad genética alta en poblaciones densas y una diferenciación genética entre ambas).

La presencia del sistema de auto-incompatibilidad y el flujo génico entre los matorrales pueden explicar estos resultados. Los sistemas de auto-incompatibilidad genética evitan la auto-fertilización y en las especies que los presentan las tasas de entrecruzamiento son altas e indistiguibles de uno (Franklin-Tong y Franklin, 2003; Hiscock y Tabah, 2003). El flujo génico en especies que se entrecruzan tiende a ser alto, por ello la diversidad genética en estas especies es también alta. En *F. cernua* el flujo génico a través de polen es alto y además el arreglo espacial de las poblaciones (relacionado con el sistema de escorrentías) favorece una dispersión secundaria de las semillas cuando los eventos de precipitación permiten el escurrimiento superficial del agua. Tanto el flujo génico, como la presencia del sistema de auto-incompatibilidad explican porqué las poblaciones no divergen y porqué la diversidad genética es alta dentro de las poblaciones.

Adicionalmente, la escala utilizada para el análisis de la estructura genética (matorrales) puede enmascarar el efecto que tiene el agrupamiento de individuos en una escala menor específicamente el de los sitios dentro de los matorrales. En este sentido, es importante recordar que los sitios dentro de los matorrales difirieron genéticamente ($F_{ST} = 0.0807$, capítulo 3), y que presentan un efecto estadísticamente significativo en la variación de algunos componentes de adecuación femenina (e. g. la producción de frutos y de semillas viables), así como en características como la sincronía floral (capítulo 1) y la expresión de la depresión endogámica (capítulo 2). En una escala geográfica más fina, por lo tanto, el sistema reproductivo puede afectar tanto la diversidad genética como la adecuación de *F. cernua.*

La diversidad genética (heterocigosis) fue mayor a la que se registra en varias especies de Asteraceae (capítulo 3). De hecho las F_{IT} y F_{IS} fueron negativas, lo que indica que las plantas heterócigas tienen una mayor adecuación que las plantas homócigas. Encontramos, además, que la depresión endogámica afecta la viabilidad de las semillas (capítulo 2). Estos datos nos sugieren que la selección natural está favoreciendo a los individuos heterócigos. Se ha propuesto que en ambientes áridos la heterocigosis es favorecida por selección natural, puesto que los individuos heterócigos tienen una mejor adecuación al enfrentarse a situaciones de estrés y de inestabilidad ambiental (Nevo, 1988).

Nuestra revisión de la variación en la diversidad genética de la familia Asteraceae apoya también la hipótesis de que la heterocigosis es favorecida por selección natural (Introducción). Sin embargo, para comprobarla en *F. cernua* se requieren más trabajos que combinen las metodologías demográficas y genéticas con un diseño enfocado a detectar diferencias en la fecundidad y supervivencia de las plantas heterócigas y homócigas; así como estudios que utilicen el método comparativo para valorar la diversidad genética en diversas poblaciones a lo largo del área de distribución de la especie.

En este sentido, un análisis de correlación del genotipo multilocus y la adecuación de adultos (componente de fecundidad) puede brindarnos una primera aproximación (*e. g.* en *Astrocaryum mexicanum*, Eguiarte *et al.*, 1992). Otro análisis que brinda información es el análisis de la diversidad genética en diferentes estadios del ciclo de vida; si la selección natural favorece la heterócigosis se espera un incremento de hetérocigos en los estadios finales del ciclo de vida de la especie (*e. g.* en *Cecropia obtusifolia*, Alvárez-Buylla y Garay 1994). Finalmente para probar si existe una relación entre el grado de áridez y la heterocigosis se puede estimar la diversidad genética de diferentes poblaciones a lo largo del área de distribución geográfica de *F. cernua.*

Conclusiones

La ecología reproductiva estuvo dominada mucho tiempo por el paradigma de que los sistemas de apareamientos mixtos eran una excepción y la regla era encontrar plantas que se auto-polinizan o plantas con polinización cruzada (Barret, 2003). Es en fechas recientes que se reconoce que los sistemas de apareamiento mixto son una regla, más que la excepción (Barret, 2003). De acuerdo con esta concepción, era difícil pensar que existiera una variación en los niveles de auto-incompatibilidad en las especies de las familias que presentan estos sistemas genéticos que impiden la auto-fertilización (Stephenson *et al.*, 2000). En la actualidad existen trabajos que documentan una variación continúa en los niveles de auto-incompatibilidad en las siguientes especies de Asteraceae: *Aster furcatus* (Reinart y Les, 1994), *Senecio squalidus* (Hiscock, 2000) y *Crepis sancta* (Cheptou *et al.*, 2002).

Nuestro trabajo apoya la idea de que el sistema de auto-incompatibilidad no es una característica del sistema reproductivo que se exprese de manera discreta (Stephenson *et al.*, 2000). En este sentido el conocimiento que generamos sobre la biología reproductiva de la especie estudiada constituye una aportación novedosa que nos permitió proponer hipótesis de trabajo para investigaciones futuras sobre la evolución del sistema de auto-incompatibilidad.

Encontramos además que el sistema de auto-incompatibilidad limita la adecuación de *F. cernua* y explica en parte la baja producción de semillas viables de esta especie. La presencia de un rompimiento parcial del sistema de auto-incompatibilidad asegura la reproducción sexual de la especie, pero conlleva también a la expresión de la depresión endogámica en la aborción de frutos y la baja viabilidad de las semillas.

La densidad poblacional tiene un efecto benéfico sobre la adecuación de la especie, que se expresa como una menor depredación pre-dispersión de los frutos y una mayor producción de frutos en sitios densos. La similitud entre los matorrales densos y difusos con respecto a los otros componentes de adecuación (*i. e.* producción de semillas y viabilidad), las tasas de polinización y las frecuencias alélicas se explican si consideramos las restricciones que impone el sistema de auto-incompatibilidad y el flujo génico entre ambos matorrales. El sistema de auto-incompatibilidad es un factor limitante en la adecuación de *F. cernua*, sin embargo, la variabilidad en la expresión de este sistema permite que la reproducción sexual se lleve a cabo en la especie. En este sentido, la presencia de un sistema de entrecruzamiento mixto brinda ventajas ecológicas a especies que como *F. cernua* tienen un potencial reproductivo limitado.

Diagrama 1

Eventos fenológicos involucrados en el ciclo de la reproducción sexual de una planta

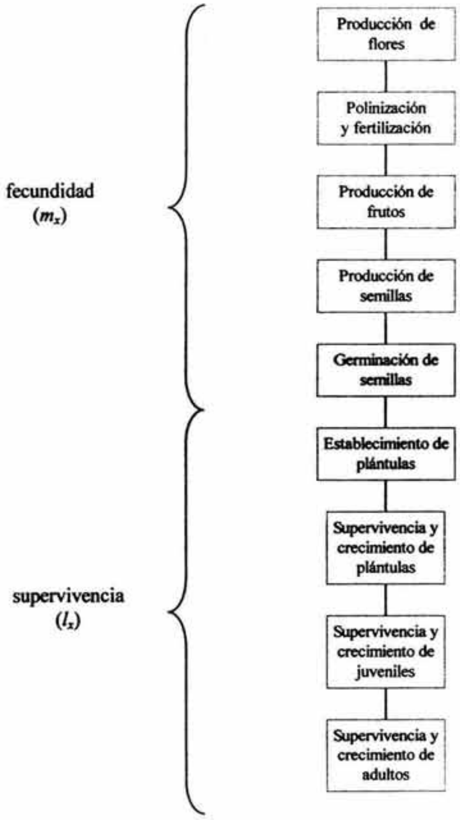
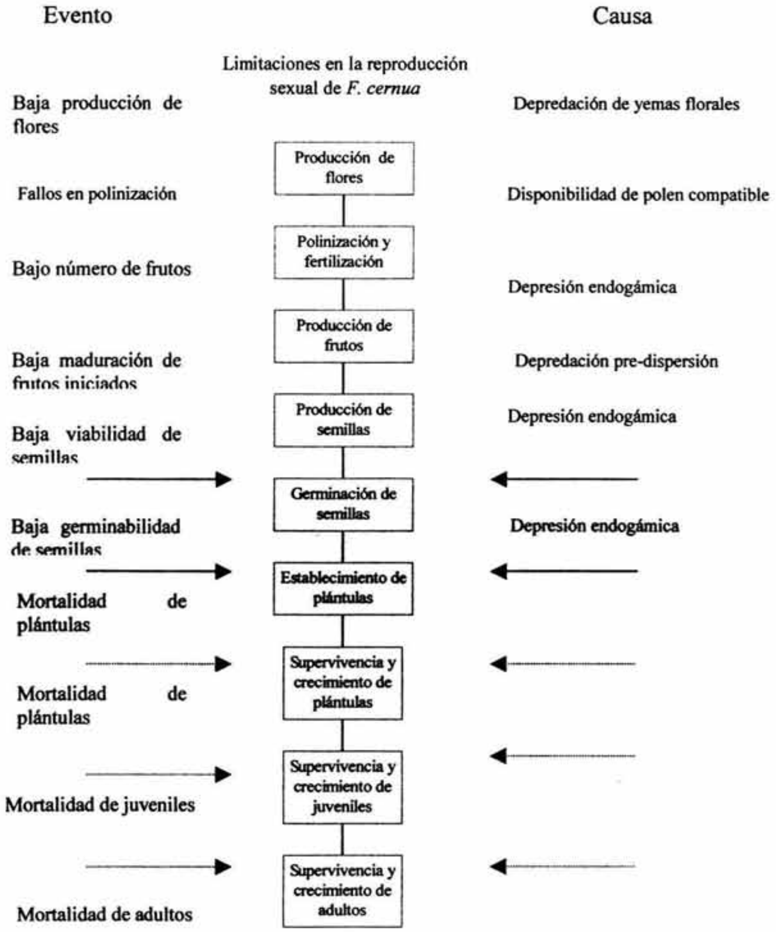


Diagrama 2


Evento
Causa
Limitaciones en la reproducción sexual de *F. cernua*
Baja producción de flores
Depredación de yemas florales
Producción de flores
Fallos en polinización
Disponibilidad de polen compatible
Polinización y fertilización
Bajo número de frutos
Depresión endogámica
Producción de frutos
Baja maduración de frutos iniciados
Depredación pre-dispersión
Producción de semillas
Depresión endogámica
Baja viabilidad de semillas
Germinación de semillas
Baja germinabilidad de semillas
Depresión endogámica
Establecimiento de plántulas
Mortalidad de plántulas
Supervivencia y crecimiento de plántulas
Mortalidad de plántulas
Supervivencia y crecimiento de juveniles
Mortalidad de juveniles
Supervivencia y crecimiento de adultos
Mortalidad de adultos

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