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**Diferenciación y flujo génico entre poblaciones de
Polaskia chichi con distintos niveles de domesticación
en zonas áridas del centro de México**

TESIS

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PRESENTA
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COORDINACIÓN

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Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 01 de marzo de 2004, se acordó poner a su consideración el siguiente jurado para el examen de grado del Doctorado en Ciencias del alumno(a) **Marta Adriana Otero Arnaiz**, con número de cuenta 82225045 y número de expediente , con la tesis titulada: **"Diferenciación y flujo génico entre poblaciones de Polaskia chichipec con distintos niveles de domesticación en zonas áridas del centro de México."**, bajo la dirección del (la) **Alejandro Casas Fernández**.

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Resumen.

En esta tesis se estudió el efecto evolutivo del manejo tradicional de poblaciones de *Polaskia chichipe* en el Valle de Tehuacan. Se estudió la biología de la polinización, sistemas de apareamiento, y fenología; la estructura genética y flujo génico entre poblaciones silvestres, manejadas *in situ* y cultivadas de *Polaskia chichipe* y la información generada se analizó y se integró con el análisis información etnobotánica y morfológica generada en estudios previos. En los estudios de biología reproductiva se encontraron barreras reproductivas parciales al intercambio de polen entre poblaciones silvestres, silvícolas y cultivadas. Se encontraron barreras temporales parciales producidas por cambios en la fenología floral de las poblaciones sujetas a diferente manejo, así como barreras espaciales parciales dadas por un limitado movimiento de polen en un área de 40 m. En cuanto al sistema de apareamiento, se encontró que *Polaskia chichipe* presenta un elevado entrecruzamiento y baja autopolinización, y al parecer los humanos han seleccionado individuos con autopolinización, ya que éstos son más abundantes en las poblaciones manipuladas (43 – 62%) con respecto a las silvestres (17 – 19%). Además se encontraron cambios fisiológicos en cuanto a la germinación de la especie, siendo ésta más rápida y con una tasa significativamente mayor ($P = 0.02$) en las poblaciones manipuladas, tanto cultivadas (77.2%) como bajo manejo silvícola (76.3%), que en las poblaciones silvestres (60.8%). Las barreras reproductivas parciales encontradas explican en parte el mantenimiento de la variación morfológica y fisiológica observada. La identidad de las poblaciones se ha mantenido por las barreras reproductivas parciales y por una continua selección artificial. La variación genética de *Polaskia chichipe* fue de $H_T = 0.658$, pero la diferenciación entre poblaciones fue baja ($F_{ST} = 0.015$). Al analizar la variación genética entre poblaciones bajo diferente manejo se encontró una mayor diversidad y menores índices de fijación en las poblaciones silvestres ($H_E = 0.631 \pm 0.031$; $F_{IS} = 0.07$), la menor variación se presentó en las poblaciones silvícolas ($H_E = 0.51 \pm 0.05$, $F_{IS} = 0.17$) y los valores intermedios en las poblaciones cultivadas ($H_E = 0.56 \pm 0.03$; $F_{IS} = 0.14$), con una diferenciación genética baja, pero significativa entre poblaciones bajo diferente manejo ($F_{ST} = 0.005$, $P < 0.04$). Un patrón de aislamiento por distancia ($r^2 = 0.314$, $P < 0.001$) con migración entre poblaciones vecinas con diferente tipo de manejo parece haber determinado poblaciones más homogéneas contrarrestando los efectos de la selección artificial. Lo anterior se corrobora en el estudio del flujo génico, en el cual se encontró que el 30% del flujo de polen es externo a la población de referencia, lo que contribuye a homogeneizar las poblaciones. Por otro lado, la estructura genética espacial responde al modelo de aislamiento por distancia no sólo a nivel poblacional, sino también en el análisis de los individuos dentro de las poblaciones y dentro de la metapoblación. Las estimaciones directas a partir del análisis de paternidad e indirectas a partir del modelo de aislamiento por distancia del tamaño de vecindad son análogas (74 a 205 individuos), lo que sugiere que la estructura genética de *Polaskia chichipe* representa un estado de equilibrio entre migración y deriva génica. El manejo tradicional de esta especie ha determinado cambios en la morfología, fisiología y fenología, pero el elevado flujo génico está contrarrestando el efecto de la selección artificial, por lo que el proceso de domesticación en *P. chichipe* se encuentra en un estado incipiente.

Palabras clave: Recursos genéticos, biología integrativa, *Polaskia chichipe*, cactáceas columnares, domesticación, marcadores genéticos, flujo génico

Abstract

Evolutionary effects of the traditional management of *Polaskia chichipe* populations were studied in the Tehuacan Valley. Pollination biology, breeding system, phenology, genetic structure and gene flow among wild, silvicultural managed *in situ* and cultivated populations were studied and the information was analyzed along with ethnobotanic and morphological knowledge generated in previous studies. Studies on reproductive biology found partial reproductive barriers to pollen interchange among wild, silvicultural and cultivated populations. Partial temporal barriers produced by changes in the floral phenology of the populations under different management, as well as partial spatial barriers produced by a limited pollen movement in an average area of 40 m, were documented. Breeding system of *Polaskia chichipe* had a high outcrossing and a low selfpollination. Apparently humans have favored individuals with selfpollination, since these are more abundant in manipulated populations (43 – 62%) than in wild populations (17 – 19%). Physiological changes were found in germination, which was faster with a significant ($P = 0.02$) high rate in manipulated populations (77.2% and 76.3%, in cultivated and in silvicultural populations, respectively), than in wild populations (60.8%). Partial reproductive barriers could explain in part the morphological and physiological variation observed. The identity of the populations has been maintained by these partial reproductive barriers and by a continual artificial selection. The genetic variation of *Polaskia chichipe* was $H_T = 0.658$, but the differentiation of populations was low ($F_{ST} = 0.015$). The analysis of the genetic variation among populations under different management type showed a higher diversity and lower endogamy coefficient in wild populations ($H_E = 0.631 \pm 0.031$; $F_{IS} = 0.07$) The lowest variation was observed in silvicultural populations ($H_E = 0.51 \pm 0.05$, $F_{IS} = 0.17$), and intermediate values were recorded in cultivated populations ($H_E = 0.56 \pm 0.03$; $F_{IS} = 0.14$), with a low, but significant differentiation among populations under different management type ($F_{ST} = 0.005$, $P < 0.04$). A pattern of isolation by distance ($r^2 = 0.314$, $P < 0.001$) with migration among neighbor populations with different management type seems to have determined more homogeneous populations balancing the effects of the artificial selection. This result was corroborated by the study on gene flow, since at least 30 % of pollen flow is immigrant to the reference population, contributing to the homogenization of the populations. Additionally, the genetic spatial structure responds to the isolation by distance model, not only at population level, also in the analysis of individuals within populations and within the metapopulation. Direct estimations through paternity analysis and indirect estimations through the model of isolation by distance of neighborhood size are similar (75 to 105 individuals), suggesting that this genetic structure is representative of an equilibrium state. Apparently, traditional management processes have promoted changes in morphology, phenology, and physiology, but gene flow is counteracting the effect of domestication to some degree, and therefore, domestication of *Polaskia chichipe* is in an incipient stage.

Key words: Genetic resources, integrative biology, *Polaskia chichipe*, columnar cactus, genetic markers, gene flow, domestication

INTRODUCCION GENERAL

Domesticación de cactáceas columnares en México

La domesticación es un proceso evolutivo que involucra cambios genéticos asociados a características morfológicas, fisiológicas y de comportamiento en poblaciones de plantas manipuladas por los seres humanos, con respecto a las poblaciones silvestres de las cuales se originan (Helbaek 1959; Harris 1972; Doebley 1992; Harlan 1992). En este proceso, la selección artificial interviene como fuerza evolutiva principal, favoreciendo la supervivencia y reproducción de fenotipos que presentan características ventajosas para los seres humanos; esto es, individuos con mejores características para ser utilizadas como alimento, medicina u otros usos (Zohary 1984; Harlan 1992). Aunque la domesticación está dirigida principalmente por la selección artificial, la selección natural puede seguir influyendo en la evolución de las plantas bajo domesticación.

La domesticación es un proceso relativamente reciente en la historia de la humanidad, pues en general se encuentra asociado a las prácticas agrícolas, cuya antigüedad se estima en no más de 12,000 años en Medio Oriente (Zohary y Hopf 1994) y en alrededor de 10,000 años en Mesoamérica (MacNeish 1992). En sus niveles más avanzados, las plantas alcanzan una total dependencia del hombre para sobrevivir y reproducirse (Hawkes 1983). Sin embargo, la domesticación es un proceso continuo y aún en sus etapas más avanzadas la domesticación sigue operando, generando nuevas variantes de acuerdo con las variaciones ambientales y culturales, espaciales y temporales a las que continuamente las somete la cultura humana. A través de estos procesos se pueden encontrar diferentes grados de domesticación que parecen relacionarse con distintos grados de intensidad con los que opera el proceso o a la

antigüedad con la que éste se ha llevado a cabo (Hillman y Davies 1990; Casas y Caballero 1996; Casas et al. 1997b; 1998; 1999c).

Se han propuesto tres modelos para explicar la evolución de plantas bajo domesticación. Cada uno de ellos trata de explicar diferencias en cuanto a los tiempos de la domesticación, el tipo de especies en las que opera y a las implicaciones biológicas y culturales del proceso. Estos modelos se basan en evidencias arqueológicas y biológicas. El modelo de semicultura trata de explicar la domesticación a partir del cultivo de generaciones sucesivas de plantas propagadas por semillas (Harris 1972; Hillman y Davies 1990; Harlan 1992). El modelo de vegecultura sugiere que la domesticación surgió a partir de la selección y cultivo de rizomas, tubérculos, tallos subterráneos y otras estructuras vegetativas (Harris 1972). En el caso de árboles con propagación vegetativa, este modelo propone que el proceso seleccionó a los individuos excepcionalmente buenos en cuanto a las características del fruto (Zohary y Spiegel-Roy 1975). Ambos modelos proponen que el cultivo es una condición necesaria para la domesticación. Sin embargo, el modelo de silvicultura, propuesto más recientemente (Casas et al. 1997a), sostiene que algunas plantas pueden ser domesticadas *in situ* a partir del manejo de poblaciones silvestres, mediante el cual se toleran, promueven y protegen fenotipos favorables, mientras que los fenotipos no deseados pueden ser eliminados. Este modelo ha resultado útil para explicar la domesticación de plantas perennes de ciclo de vida largo. Los autores de este modelo sugieren que esta forma de manipulación de plantas podría haber surgido incluso antes que el cultivo en Mesoamérica y que el

manejo de las especies *in situ* facilitó su posterior manejo *ex situ* (Casas et al. 1994; 1997a).

Los autores del modelo de silvicultura sostienen que éste puede explicar la domesticación de diversos árboles mesoamericanos, cactáceas columnares con propagación vegetativa como *Stenocereus stellatus* (Casas et al. 1998; 1999b), y posiblemente también de otras cactáceas columnares. En parte, el presente estudio está encaminado a generar evidencia empírica para poner a prueba este modelo. Si la silvicultura ha sido importante en el proceso de domesticación de cactáceas en Mesoamérica, entonces deben haber habido diferentes tipos de interacciones o formas de manejo *in situ*, que determinaron cambios morfológicos, fisiológicos, fenológicos y genéticos con respecto a las poblaciones silvestres. Por otro lado, estas formas de manejo deben tener un fuerte componente selectivo por parte del hombre, ya que al encontrarse *in situ* las poblaciones manejadas se encuentran en contacto con las poblaciones silvestres y pueden llevar a cabo intercambio genético haciendo difícil la fijación de los caracteres deseados por el hombre.

Los estudios arqueológicos del Valle de Tehuacán (MacNeish 1967) y de Guilá Naquitz, Oaxaca (Flannery 1986) sugieren que desde la prehistoria, los pueblos que habitaron el área han utilizado diversas especies de cactáceas y que éstas se encuentran entre los elementos vegetales más importantes de la subsistencia humana, probablemente debido a su abundancia y diversidad, y debido a que la mayor parte de ellas producen partes comestibles (Casas y Barbera 2002).

Se han descrito alrededor de 850 especies de cactáceas dentro del territorio mexicano y cerca de 420 especies para el área mesoamericana (Bravo-Hollis 1978), mientras que en una revisión reciente de los estudios etnobotánicos efectuados en la porción mexicana de Mesoamérica se documentan un total de

118 especies de cactáceas utilizadas por los pueblos indígenas (Casas y Barbera 2002; Casas et al. en prensa). Entre las cactáceas columnares Casas y Barbera (2002) reportan 43 especies, de las cuales cerca de la mitad son “gigantes” algunas de ellas de cerca de 15 m de alto, con lento crecimiento vegetativo, floreciendo por primera vez hasta después de varias décadas de crecimiento, y no son cultivadas (Casas et al., 1999a). Pero otras 23 especies son relativamente más pequeñas, miden de 2 a 8 m de alto, crecen más rápidamente que las “gigantes” (la primera floración puede ocurrir después de 6 a 8 años después de la germinación de las semillas, o de 2 a 4 años después de haber sido propagadas vegetativamente), la mayor parte de ellas se propaga vegetativamente y pueden ser cultivadas (Casas y Barbera, 2002). En esta misma revisión se destaca que las cactáceas son usadas principalmente por sus frutos comestibles. Los frutos de todas las cactáceas columnares son comestibles, pero pueden distinguirse: 1) especies que producen frutos dulces y jugosos, que se consideran como “frutos de buena calidad” y son las especies más comúnmente recolectadas en condiciones silvestres o cosechadas bajo cultivo; 2) especies cuyos frutos se consideran como de “calidad regular”, y que son colectados sólo ocasionalmente debido a que son demasiado altos para su obtención, o porque presentan espinas abundantes y largas, o porque no son sabrosos (agrios o insípidos), y 3) especies cuyos frutos no contienen pulpa jugosa y se consumen sólo durante periodos de escasez de alimentos, y que son considerados frutos de “mala calidad” (Casas y Barbera, 2002)

Además del consumo humano de los frutos, los tallos y los frutos de todas las especies son utilizados como forraje, particularmente para cabras, vacas y burros. Los pastores también suelen recolectar frutos o sus cáscaras para dárselas directamente al ganado o para

preparar ensilados. Con los frutos de algunas especies se prepara una bebida fermentada conocida como *nochoctli* o *colonche*, a manera de pulque o vino. Las semillas de algunas especies se consumen separadas de la pulpa. Los botones florales de varias especies también son comestibles. Los tallos tiernos de algunas especies también son comestibles. Generalmente se quitan las espinas y se hacen cortes longitudinales a la rama con el fin de eliminar la porción medular. La madera de varias especies de cactáceas columnares gigantes se utiliza comúnmente en la construcción de techos y cercas de las casas campesinas tradicionales. Algunas especies se utilizan como cercas vivas y como bordos de contención en terrazas (Casas et al. 1997a; 1999a; 2002). Los tallos secos de algunas especies se utilizan como leña para calentar y preparar alimentos, y los tallos de *Polaskia chichipe*, *P. chende* y *Stenocereus stellatus* son además fuente de leña para la manufactura de cerámica tradicional.

La recolección de productos útiles es una práctica común en las poblaciones silvestres de todas las especies. En general, la recolección de frutos ocurre de manera selectiva, la gente prefiere los frutos de las especies o de las variantes de una especie que tienen pulpa jugosa, mayor tamaño, en ocasiones los frutos más dulces, en otras los más ácidos, dependiendo del destino del producto. Para elaborar mermeladas se prefieren los agrios, para consumo directo, los frutos con cáscara delgada o los de cáscara gruesa si se piensa comercializarlos, ya que la cáscara gruesa es más resistente a la pudrición (Casas et al. 1997a). Sin embargo, es posible observar otro tipo de interacciones en algunas de las especies. Por ejemplo, las poblaciones silvestres de algunas especies son sujetas a formas de silvicultura. Por medio de este manejo, los campesinos dejan en pie algunos individuos cuando talan la vegetación, y en ocasiones también promueven su

abundancia por medio de propagación vegetativa (Casas y Barbera, 2002).

En el caso de *Polaskia* spp. y *Escontria chiotilla* la plantación de ramas, aunque no imposible, es rara pues la propagación vegetativa es menos exitosa que en los casos de *Stenocereus*. Más bien, la gente practica el trasplante de individuos jóvenes (de entre 20 y 30 cm) con el fin de optimizar el uso del espacio abierto para fines agrícolas (Arellano y Casas 2003; Carmona y Casas en prensa). Debido a que los individuos así tolerados compiten por espacio con las plantas cultivadas, la gente selecciona cuáles especies son las mejores para tolerarse, de acuerdo con su utilidad, así como cuáles individuos son los mejores para tolerarse, de acuerdo con la calidad de sus frutos. La gente generalmente prefiere dejar en pie a los individuos con frutos relativamente más grandes, con sabor más dulce, con cáscara delgada y con pocas espinas.

Finalmente, se practica el cultivo en las huertas y solares. La gente selecciona los fenotipos deseables tanto en poblaciones silvestres como en individuos previamente cultivados, corta sus ramas y las planta en el sitio conveniente. Los individuos que se encuentran en las huertas también pueden derivarse de plántulas toleradas por los campesinos, las cuales se establecieron en las huertas a partir de semillas dispersadas a través de excretas de aves, murciélagos o humanos o a partir del trasplante de individuos jóvenes, los cuales aportan nueva variación genética junto con las plántulas que se establecen y se toleran. Debido a que la gente no reconoce a las diferentes variantes de frutos con base en características vegetativas, la decisión de eliminar o dejar en pie a los individuos establecidos por esta vía se toma hasta después de que los individuos producen frutos por primera vez (Casas y Barbera, 2002).

Aunque se han registrado 12 especies de cactáceas columnares cultivadas (Casas y Barbaera,

2002), hasta el presente los únicos casos analizados bajo la perspectiva de procesos de domesticación son *Stenocereus stellatus*, *S. queretaroensis*, *S. pruinosus*, *S. fricci*, *Escontria chiotilla*, *Polaskia chichipe* y *P. chende* (Pimienta-Barrios y Nobel 1994; Rebollar et al. 1997; Casas et al. 1999b; 1999c; Luna 1999; Cruz y Casas 2002; Arellano y Casas 2003; Otero-Arnaiz et al. 2003; Tinoco et al. 2004; Carmona y Casas en prensa; Oaxaca-Villa et al. en prensa). *Stenocereus* es el género con más amplia distribución. Las especies de *Stenocereus* referidas se distribuyen en dos a seis de las provincias florísticas reconocidas por Rzedowski (1978), *E. chiotilla* es la única especie del género y se distribuye en tres provincias, mientras que las dos especies del género *Polaskia* son endémicas del Valle de Tehuacán-Cuicatlán (Dávila-Aranda et al. 2002). En general, estas especies se encuentran en condiciones silvestres formando parte de bosques tropicales deciduos y matorrales xerófitos, frecuentemente siendo componentes dominantes de la vegetación y determinando asociaciones vegetales específicas (Valiente-Banuet et al. 2000). Para todas las especies se han registrado poblaciones con manejo silvícola y todas, excepto *E. chiotilla* y *P. chende*, son cultivadas (Casas et al. 1999a).

Las siete especies mencionadas presentan una considerable variación morfológica, especialmente en las características de sus frutos, la que en parte es determinada genéticamente y en parte por el ambiente en que se encuentran. Aparentemente, esta variación morfológica es el resultado de la selección artificial en adición a la influencia de la selección natural y otras fuerzas evolutivas. Esclarecer tales componentes y origen de la variación constituye un aspecto central de las investigaciones del proceso de domesticación. Los estudios morfométricos efectuados han buscado analizar los patrones de variabilidad morfológica de las

diferentes especies en distintas condiciones ambientales y bajo distintas formas de manejo humano, con el fin de examinar en qué medida la variación morfológica puede estar relacionada con factores ambientales, genéticos y en qué medida por la intervención humana (Casas et al. 1999b; Cruz y Casas 2002; Arellano y Casas 2003; Carmona y Casas en prensa).

El color de la pulpa, el sabor, la cantidad de material comestible, el grosor y la cantidad de espinas de los frutos, son las principales características usadas por la gente en la clasificación folk de las variantes dentro de las especies, en la asignación de calidad a los productos, y en la selección de los individuos que determina una propagación diferencial de algunas variantes (Pimienta-Barrios y Nobel 1994; Casas et al. 1997b; 1999a; Luna 1999; Arellano y Casas 2003; Carmona y Casas en prensa). Este manejo, entonces, parece estar involucrando selección artificial, que parece ser particularmente intensa en los solares y huertos indígenas, en donde la manipulación de estas cactáceas se lleva a cabo mediante una continua plantación y reemplazo de individuos. Pero la selección artificial también parece ser significativa bajo manejo *in situ*, en el que la selección se dirige principalmente a aumentar las frecuencias de los fenotipos más favorables existentes en las poblaciones silvestres (Casas et al. 1999b).

Los estudios comparativos de la biología reproductiva en poblaciones silvestres, manejadas *in situ* y cultivadas han permitido analizar si ha sido modificada por la domesticación. Además, se ha analizado si tales modificaciones determinan barreras al flujo de genes entre las poblaciones para entender así los mecanismos biológicos que permiten el mantenimiento de las divergencias morfológicas encontradas. Los estudios de biología reproductiva muestran que en *S. stellatus* y *S. pruinosus* la antesis es nocturna y los polinizadores son

murciélagos (*Leptonycteris curasoe* y *L. nivalis*, principalmente, de acuerdo con Casas *et al.*, 1999c). La polinización por murciélagos sugiere que existe un marcado movimiento de polen entre poblaciones, ya que estos animales son capaces de volar entre 30 y 100 km en una noche para alimentarse de néctar y frutos de cactus columnares. El aislamiento por distancia, dentro de cada región, entre poblaciones silvestres, manejadas *in situ* y cultivadas es entonces improbable, ya que las distancias entre las poblaciones estudiadas es del orden de no más de 10 km. Adicionalmente, los estudios fenológicos han encontrado que la floración en todas las poblaciones tiene un traslape de al menos 75 días, indicando que las barreras temporales también son improbables.

En el caso de *Escontria chiotilla* y *Polaskia* spp., la antesis es diurna y los polinizadores son principalmente las abejas *Xylocopa mexicanorum* y *Apis mellifera*, así como los colibríes *Amazilia violiceps*, *Cinanthus sordidus* y *C. latirostris* (Cruz y Casas 2002; Otero-Arnaiz *et al.* 2003; Oaxaca-Villa *et al.* en prensa). En estos casos, al igual que en los anteriores, las distancias entre poblaciones se encuentran dentro de los radios en que pueden moverse los polinizadores; sin embargo, es más frecuente que su forrajeo se lleve a cabo entre flores del mismo individuo o de individuos cercanos (observación personal). En *E. chiotilla* y *P. chende* hay traslapes en los periodos de floración, por lo que el aislamiento temporal es improbable. Sin embargo, en el caso de *Polaskia chichiipe*, como se verá en el capítulo I de esta tesis, existe un desplazamiento temporal en los picos de floración entre poblaciones con diferente manejo. Las poblaciones silvestres tienen su pico de floración en febrero, las manejadas *in situ* en marzo y las cultivadas en abril. Además, cuando se presenta el pico de floración en las cultivadas, la floración ha terminado en las silvestres. Estas

observaciones sugieren que existen barreras temporales parciales al flujo de polen en esta especie.

Otro factor que pudiera determinar aislamiento reproductivo entre poblaciones con diferente manejo sería una capacidad diferencial de germinación y sobrevivencia de plántulas derivadas de individuos cultivados bajo condiciones silvestres. Hasta el momento se ha estudiado el comportamiento germinativo de *S. stellatus* y *P. chichiipe* (Rojas-Aréchiga *et al.* 2001; Otero-Arnaiz *et al.* 2003). En ambos casos se ha documentado que las semillas derivadas de individuos de poblaciones manipuladas por el hombre germinan en mayor porcentaje y con mayor rapidez. Estos resultados indican que la domesticación ha determinado consecuencias en el comportamiento germinativo de las semillas, pero no permiten concluir acerca del papel de tales diferencias en el establecimiento de barreras reproductivas. Hace falta aún efectuar experimentos sobre el éxito en el establecimiento de plántulas de origen cultivado en condiciones silvestres y viceversa.

La información etnobotánica indica que los pueblos mesoamericanos usan y manejan un considerable número de especies de cactáceas. También indica que la gente toma decisiones sobre cómo manipular a estas plantas de acuerdo con la calidad de sus productos y de acuerdo con el papel que juegan en la subsistencia. De este modo, las especies y variantes cultivadas o manejadas *in situ* son generalmente aquellas con las características más útiles de sus frutos. Sin embargo, el cultivo es particularmente intensivo en aquellas áreas en donde la comercialización de frutos o su consumo por las unidades familiares hace necesario producir más y mejores frutos (Casas *et al.* 1997b). La disponibilidad de recursos también parece ser crucial; por ejemplo, Casas *et al.* (1997b) encontraron que *S. stellatus* se cultiva intensamente en lugares en donde las poblaciones silvestres son escasas, pero no en donde son

abundantes. La viabilidad y facilidad de manipulación también parece ser significativa. Aunque especies “gigantes” como *Neobuxbaumia tetezo*, *N. mezcalaensis*, *Mitrocereus fulviceps* y *Pachycereus weberi*, entre otras, producen frutos de muy buena calidad, no se cultivan debido a que su lento crecimiento hace poco atractivo invertir esfuerzos de plantar semillas y cuidar plántulas y plantas juveniles sin obtener una retribución por décadas. El lento crecimiento puede no ser relevante para tomar decisiones en cuanto al manejo de poblaciones silvestres de estas especies *in situ*, pues bajo esta forma de manejo la gente simplemente tolera la presencia de algunas plantas durante los aclareos de vegetación. Sin embargo, el efecto de la selección artificial favoreciendo fenotipos particulares de estas especies *in situ* podría ser imperceptible dadas las dificultades que tiene la gente para aumentar la frecuencia de los fenotipos deseables por propagación intencional directa. Además, cuando se siembran semillas que derivan de fenotipos deseables, la varianza genética aditiva de los rasgos deseables hace incierto que los fenotipos seleccionados sean aquellos que se expresen en la progenie. En contraste, la fijación de caracteres deseables es relativamente fácil en especies con propagación vegetativa tales como *S. stellatus*, *S. pruinosus* o *S. queretaroensis*.

La selección artificial se lleva a cabo aparentemente identificando primeramente los individuos deseables y posteriormente aumentando su disponibilidad mediante propagación vegetativa, pero también a través de dejar en pie y proteger las formas deseables. Esta información sugiere que la selección artificial es un proceso actual o potencial que puede aplicarse a plantas de fácil manejo tales como *Stenocereus griseus*, *S. stellatus*, *S. pruinosus*, *S. queretaroensis* y *S. fricii* como se ha documentado por Pimienta-Barrios y Nobel (1994), Casas et al. (1997b) y

Rebollar et al. (1997). Pero la selección artificial parece ser significativa en especies que son intensamente manejadas *in situ* y que presentan una importante variación morfológica en caracteres que son blanco de la selección artificial, tales como *Escontria chiotilla*, *Polaskia chichipe* y *P. chende* (Cruz y Casas 2002; Arellano y Casas 2003; Carmona y Casas en prensa). Este podría ser también el caso de *Myrtillocactus geometrizans*, *M. schenki*, *Pachycereus hollianus* y *P. marginatus* (Casas et al. 1999a). Los estudios de caso desarrollados hasta ahora proporcionan modelos de selección artificial *in situ* y *ex situ* que ayudan a analizar los patrones de domesticación de estos grupos de especies y quizás de otras plantas mesoamericanas. Sin embargo, es importante establecer que la selección artificial a favor de una mayor abundancia de fenotipos con mejores frutos y mayor productividad ha tenido un efecto sobre el sistema de apareamiento, promoviendo la abundancia de individuos auto-compatibles (más productivos), así como sobre los niveles y estructura de la variación genética de la especie. Esta información resulta crucial para entender cómo se mantiene la divergencia morfológica y qué factores están teniendo una mayor influencia en el proceso de domesticación de cada especie en particular y establecer las generalizaciones posibles.

El presente es el primer trabajo en el que se analiza en una especie perenne cómo se ha mantenido la variación morfológica asociada a la domesticación, las barreras reproductivas, los niveles de divergencia genética e importancia del flujo génico entre poblaciones simpátricas bajo diferentes niveles de manejo. La información que se deriva del estudio aspira a aportar empírica que pruebe el modelo de manejo silvícola para explicar la evolución bajo domesticación.

Polaskia chichipe es una cactácea columnar endémica del centro de México, específicamente del

Valle de Tehuacán. Crece de manera silvestre como parte del matorral xerófilo denominado “chichipera” (Valiente-Banuet et al. 2000), el cual se encuentra fuertemente asociado a suelos derivados de rocas volcánicas entre los 1600 y 2300 m snm. Algunas poblaciones silvestres se encuentran bajo manejo *in situ* y se cultivan principalmente en huertas y solares tradicionales (Casas et al. 1999a).

Es conocida popularmente como “chichipe” o “chichibe”, al fruto se le llama “chichituna” o “chichitun”. Según la clasificación chocho popoloca, esta especie se conoce por el nombre de “túchi-cásha”, y según la clasificación náhuatl como “tepequio nochtli” (Casas et al. 1999a). Según la clasificación mixteca la especie se denomina como “tnu dichitun” o “too dichitun”. Es una de las cactáceas columnares de la región con mayor importancia económica. El fruto es la parte de la planta más valorada; éste se consume fresco o seco y es usado para la elaboración de mermeladas, las cuales son comercializadas. Las cáscaras del fruto y ramas son utilizadas como forraje. Las ramas son utilizadas también como combustible, particularmente en hornos para la producción de cerámica (Casas et al. 1999a).

Polaskia chichipe es una de las cactáceas columnares de la región en las que se han identificado los tres niveles de interacción mencionados anteriormente: 1) recolección en poblaciones silvestres, 2) poblaciones con manejo silvícola y 3) poblaciones cultivadas (Casas y Barbera 2002). Carmona y Casas (en prensa) reportan diferenciación morfológica entre los individuos de poblaciones silvestres, manejadas *in situ* y cultivadas. Estas diferencias reflejan que la selección artificial parece haber tenido una influencia significativa sobre la estructura fenotípica de las poblaciones y que tal selección actúa tanto en poblaciones con manejo silvícola como en poblaciones cultivadas. Tal selección favorece individuos más vigorosos, con frutos mayores,

con una mayor cantidad de pulpa y semillas en relación con los individuos de poblaciones silvestres. Sin embargo, permanece aún la incertidumbre de cuál es el efecto del ambiente en estos patrones de variación. La información sobre el sistema de apareamiento y la estructura genética de las poblaciones de esta especie, además de ser de interés para entender cómo ha sido el proceso de domesticación, es también de importancia práctica en el diseño de programas de aprovechamiento, manejo y conservación. El sistema de apareamiento es uno de los determinantes principales de cómo la diversidad genética se distribuye entre los individuos y poblaciones dentro de la especie. Cómo es la naturaleza de este sistema, en consecuencia, es información que debe tomarse en cuenta en la colección de germoplasma en programas de conservación *ex situ* (Young y Brown 1998) y, junto con información sobre los niveles de variación genética, permite decidir qué poblaciones deben ser prioritarias para la conservación *in situ* (Frankel et al. 1995).

En el contexto de la información disponible, las hipótesis que guiaron la presente investigación fueron las siguientes:

1) En numerosas especies domesticadas, los humanos han modificado el sistema reproductivo de las especies manipuladas en comparación con sus parientes silvestres. Generalmente se han favorecido sistemas auto-compatibles sobre sistemas auto-incompatibles. Ello les ha permitido asegurar su producción por vía femenina, asegurando la producción de frutos independientemente de la presencia de polinizadores. Este cambio ha favorecido el aislamiento reproductivo entre plantas domesticadas y sus parientes silvestres. Por ello, la primera hipótesis fue que la selección artificial ha favorecido individuos auto-compatibles en las poblaciones manejadas de *Polaskia chichipe*, con respecto a sus poblaciones silvestres.

2) Al parecer no existen barreras espaciales para la dispersión de polen entre las poblaciones silvestres y manipuladas, debido a que las poblaciones no son discretas y se encuentran parches de individuos de *P. chichipe* entre ellas, los cuales pueden servir de puentes para el intercambio de polen. Sin embargo, la selección sobre fenotipos con un periodo de fructificación más amplio puede alterar de manera indirecta la fenología floral creando barreras temporales para la reproducción. Además, es posible que existan también sistemas de incompatibilidad genética entre los individuos de las diferentes poblaciones, como los encontrados de manera preliminar en *Stenocereus stellatus* (Casas et al. 1999c), obstaculizando el flujo de polen entre ellas. Es posible también que este sistema de incompatibilidad esté en proceso y que la descendencia de cruza entre variantes bajo diferente manejo tenga una adecuación menor que la de cruza de individuos de poblaciones con el mismo tipo de manejo. Si es así, posiblemente se observará flujo génico entre las poblaciones con el mismo tipo de manejo y éste podrá ser menor o inexistente entre las poblaciones cultivadas y silvestres. Adicionalmente, se espera encontrar una mayor compatibilidad y/o adecuación en las cruza realizadas entre individuos del mismo tipo de población que entre individuos de poblaciones con diferentes niveles de manejo.

3) La selección artificial, cambios en la fenología y en la frecuencia de individuos auto-compatibles, podrían promover estructura genética. Considerando que: (i) las poblaciones manejadas y cultivadas se derivan de poblaciones silvestres, (ii) que la selección artificial implica esencialmente la eliminación de individuos no deseables y el favorecimiento de los deseables que se encontraban en las poblaciones silvestres originales, y (iii) que la auto-compatibilidad se encuentra presente en estas

poblaciones, es de esperarse que en las poblaciones manipuladas por el hombre se encuentre una reducción significativa de los niveles de variabilidad genética en relación con las poblaciones silvestres. No obstante, en las poblaciones cultivadas es factible esperar mayor diversidad genética que en las manejadas *in situ*, debido a que en estas poblaciones la gente puede introducir nuevas variantes tanto locales como de otros poblados, reemplazando los materiales cultivados previamente (Casas et al. 1999b).

4) Las poblaciones bajo manejo y silvestres de *P. chichipe* se encuentran de manera simpátrica y las cactáceas en general presentan un elevado entrecruzamiento por lo que el flujo génico puede ser suficiente para evitar un aislamiento genético entre estas poblaciones y contrarrestar los efectos de la selección en esta especie bajo domesticación.

Objetivos

Con el fin de probar las hipótesis anteriores, los objetivos particulares del presente estudio fueron:

1) Estimar los niveles de auto-compatibilidad en cada una de las poblaciones y determinar un componente de la adecuación (germinación) de la progenie resultante de auto-polinización y polinización cruzada.

2) Analizar si la selección artificial ha modificado los patrones reproductivos de *P. chichipe*, causando barreras al intercambio de polen entre poblaciones silvestres, con manejo silvícola y cultivadas.

3) Evaluar y comparar los patrones de variación y estructura genética de poblaciones silvestres, con manejo silvícola y cultivadas de *Polaskia chichipe*, con el fin de examinar si el manejo humano ha influenciado cambios en la estructura genética de estas poblaciones.

4) Estimar el flujo génico intra e interpoblacional y determinar si existe algún tipo de aislamiento reproductivo espacial entre las poblaciones.

En el Capítulo I se analiza la biología reproductiva de la polinización de *P. chichipe*, los visitantes florales más importantes y si el proceso de domesticación ha modificado los patrones reproductivos en esta especie, causado barreras en el intercambio de polen entre poblaciones silvestres, con manejo silvícola y cultivadas. En el Capítulo II se analiza si el manejo humano ha modificado los niveles y distribución de la variación genética en poblaciones silvestres, silvícolas y cultivadas de *P. chichipe* y que relación existe entre la variación genética y la distancia espacial. En el tercer capítulo se analizan las consecuencias del flujo génico en el proceso evolutivo de domesticación por medio de estimaciones directas e indirectas del flujo génico entre poblaciones bajo diferente manejo de *P. chichipe*. Finalmente la discusión general de la tesis integra la información generada en este y otros estudios para analizar el patrón general de domesticación de *P. chichipe*.

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EVOLUTION OF *POLASKIA CHICHIPE* (CACTACEAE) UNDER DOMESTICATION IN THE TEHUACÁN VALLEY, CENTRAL MEXICO: REPRODUCTIVE BIOLOGY¹

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Polaskia chichipe, a columnar cactus, is cultivated for its edible fruits in central Mexico. This study analyzed whether artificial selection has modified its reproduction patterns and caused barriers to pollen exchange between wild, managed in situ, and cultivated populations. Anthesis was diurnal (~16 h in winter, ~10 h in spring) as well as partly nocturnal (~12 h in winter, ~3 h in spring), and flowers were pollinated by bees, hummingbirds, and hawk moths. Manual cross-pollination was ~37–49% effective in all populations. Self-pollination was ~12% successful in the wild, but twice as successful (~22–27%) in managed and cultivated populations. Diurnal pollination was ~35–55% effective in winter and 100% in spring. Nocturnal pollination was successful only in winter (15%). Crosses among individuals were more effective within populations than among populations, including populations under a similar management regimen. The least successful crosses were between wild and cultivated populations. Flowers were produced in all populations from January to March, but flowering peaks differed by 1 mo among wild, managed, and cultivated populations and by 2 mo between wild and cultivated populations. The latter interrupted pollen exchange in May. Seeds from managed and cultivated populations germinated faster than those from wild individuals. Domestication has seemingly favored self-compatible *P. chichipe* plants with higher fruit yield, a longer period of fruit production, and faster seed germination, attributes that have resulted in partial reproductive barriers between wild and manipulated populations.

Key words: Cactaceae; columnar cacti; domestication; Mexico; *Polaskia chichipe*; reproductive biology; Tehuacán-Cuicatlán Valley.

Columnar cacti are among the main plants used by humans since they first occupied central Mexico's Tehuacán Valley nearly 10 000 yr ago (MacNeish, 1967). Currently, 20 species of these plants are sources of food in the region. People gather products of columnar cacti from wild populations, but seven species are also cultivated in home gardens. In addition, people manage wild populations in situ by enhancing the numbers of favorable plants when they clear natural vegetation. For some columnar cactus species, both cultivation and management in situ may involve domestication (Casas, Caballero, and Valiente-Banuet, 1999), which is an evolutionary process, guided by humans, through which heritable morphological or physiological variation of plant populations is molded by artificial selection (see Darwin, 1868; Harlan, 1992). Cacti fruits are the main parts used, and characteristics such as size, pulp color and flavor, peel thickness, and thorniness are considered when people select individual plants to manage in situ and cultivate. Artificial selection is intense in home gardens, where plants are continually replaced by others with better attributes, but it is also practiced in managed in situ populations, where desir-

able phenotypes are maintained and propagated during the perturbation of natural vegetation areas (Casas et al., 1997, 1999a; Casas, Caballero, and Valiente-Banuet, 1999).

Previous studies analyzed reproductive biology of columnar cacti in the context of their domestication in the Tehuacán Valley (Casas et al., 1999b; Cruz and Casas, 2002). In those cases, the authors concluded that artificial selection had not modified the species' reproductive biology and that the occurrence of both spatial and temporal barriers to pollen exchange among wild and manipulated populations was unlikely. Our study analyzed the case of *Polaskia chichipe* (Glosselin) Backeberg, endemic to the Tehuacán Valley. This cactus is markedly restricted to volcanic soils at elevations of 1600–2300 m, where it is a dominant element of the thorn-scrub forest type called "chichipera" (Valiente-Banuet et al., 2000). Some of the wild populations are under in situ management, and the species is also cultivated in home gardens (Casas, Caballero, and Valiente-Banuet, 1999). Humans propagate *chichipe* by planting branches, transplanting young plants, or sowing seeds from the desirable phenotypes (A. Carmona and A. Casas, unpublished data). More commonly, though, people tolerate seedlings and young plants of *chichipe* (derived from seeds in bird or human feces) in managed in situ populations and home gardens. They let the most vigorous young plants grow and then decide to leave or remove plants at reproductive age (≥ 10 yr old) according to the presence of favorable or unfavorable characteristics. *Polaskia chichipe* is one of the columnar cacti with relatively high economic value in the region. Its fruits are sold fresh or dry for human consumption. The species appears to be undergoing domestication through artificial selection, and this process has seemingly resulted in morphological differentiation between wild and manipulated populations. Fruits and seeds from cultivated and managed in situ individ-

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uals are larger than those from unmanaged wild populations (A. Carmona and A. Casas, unpublished data).

The purpose of our study was to examine whether human management has modified the reproductive biology of *P. chichipe* in managed in situ and cultivated populations as compared with wild populations. Bravo-Hollis (1978) described flowers of *P. chichipe* as diurnal, but no formal studies on reproductive biology have been conducted. Therefore, our first aim was to test if anthesis is diurnal and which animals are the probable pollinators. Studies on breeding systems in columnar cacti of the Tehuacán Valley have generally found that self-pollination fails to produce fruits (Casas et al., 1999b; Valiente-Banuet et al., 1996, 1997a, b). Therefore, if the reproductive pattern of *P. chichipe* is consistent with those of other columnar cacti, its breeding system would be self-incompatible. In some cultivated plant species, however, artificial selection has modified breeding systems, favoring self-compatible mutants because they give satisfactory yields even in absence of pollinators (Proctor, Yeo, and Lack, 1996). Therefore, we considered an alternative hypothesis: artificial selection has favored numbers of self-compatible plants.

Because domestication of *P. chichipe* has resulted in morphological divergence in fruit and seed size between wild and manipulated populations, it is possible that mechanisms of reproductive isolation among these populations have helped to maintain such divergence. Spatial barriers would be possible if wild and manipulated populations were separated by distances greater than those that pollinators usually travel. Temporal barriers would operate if the flowering seasons of wild and manipulated populations occurred at different times. Reproductive barriers could be present if pollination were more effective within than among populations or among populations under the same management regimen than among populations under a different regimen.

In general, seeds from cultivated plants commonly germinate faster than those from wild plants, owing to the latter's dormancy and hard seeds (Hawkes, 1983; Evans, 1996). In a study of *Stenocereus stellatus* (Pfeiffer) Riccobono, Rojas-Aréchiga, Casas, and Vázquez-Yanes (2001) found that artificial selection favored seedling vigor and rapid seed germination in cultivated variants. Because the management of *P. chichipe* involves selective recruitment of seedlings in home gardens and managed in situ populations and because artificial selection has determined more abundant phenotypes with larger seeds in these populations (A. Carmona and A. Casas, unpublished data), we hypothesized that seed germination could be influenced by the selective sparing of vigorous and dynamic emergent seedlings, and, therefore, cultivated and managed in situ plants would have seeds that germinated faster than those from wild plants.

MATERIALS AND METHODS

Study area—The study was conducted in nine populations of *P. chichipe* in the villages of San Luis Atlotitlán, Caltepec, and Los Reyes Metzontla, Puebla, in the Biosphere Reserve Tehuacán-Cuicatlán, Mexico (Fig. 1). Annual mean temperature and annual rainfall in the region are 21°C and 400 mm, respectively (Davila et al., 1993). Wild, managed in situ, and cultivated populations from the three villages were included in the analysis. Vegetation in wild populations is "chichipera" forest (Valiente-Banuet et al., 2000). The elevation range of wild populations studied was 1955–2166 m. The managed in situ populations occurred in "chichipera" areas that were open for cultivation of maize, and individuals of *P. chichipe* had been spared (elevation range: 1860–2118 m). The cultivated populations were composed of individ-

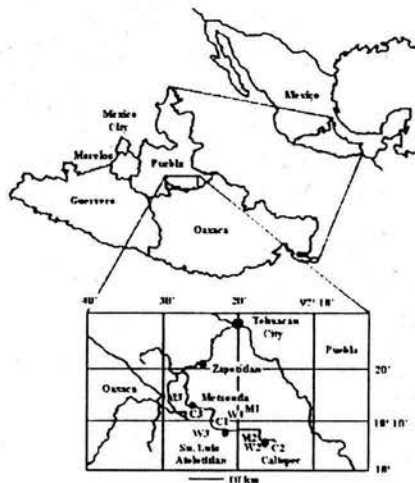


Fig. 1. Study area: the Tehuacán-Cuicatlán Valley. Populations of *Polaskia chichipe* were studied in the municipality of Caltepec, Puebla, Mexico; W = wild populations, M = managed in situ populations, C = cultivated populations.

uals in home gardens of the villages studied (elevation range: 1881–1937 m). Patches of vegetation with wild and managed individuals of *P. chichipe* existed between the populations studied.

Floral biology—Events occurring during anthesis were recorded in three populations (one of each type of management). Observations were conducted every hour on samples of 30 flowers from 6–10 individuals per population during winter (17–22 January) and in samples of 20 flowers from the same individuals during spring (26–29 March). The times of the following events were recorded: opening and closing of flowers, changes in androecia and gynoecia, pollen liberation, and turgidity of stigma. Production of nectar was measured with 5- μ L micropipettes every 2 h in samples of three flowers per recorded hour (24 previously bagged flowers per population).

Flower visitors—All insects that visited a sample of 20 flowers in each of the three populations of *P. chichipe* mentioned above were captured with entomological nets and forceps, and the time of each visit was recorded. Captured insects were preserved in 70% ethanol for later identification. Birds that visited flowers were photographed and captured with three mist nets per population. Frequency of visits of the different animal species to flowers of *P. chichipe* was recorded in the wild population of San Luis Atlotitlán in samples of 20 flowers from eight individuals in winter (23–24 January) and spring (29–30 March). The flowers were continually observed throughout anthesis. Observations were classified into 1-h intervals, and the averages of visits per flower per species were calculated per interval.

Breeding system—Field experiments were conducted in six populations (two of each management type), according to methods developed by Valiente-Banuet et al. (1996). For each of the following treatments, flower buds of at least 11 individuals per population were covered with exclusion bags just before anthesis.

1. **Nonmanipulated self-pollination**—Flower buds were left covered with exclusion bags from anthesis to the ripening or abortion of fruits. The number of individuals and flowers sampled per population type were 31 and 356 from wild, 28 and 352 from managed in situ, and 29 and 630 from cultivated.

2. **Manual self-pollination**—Pollen from a given flower was deposited on its own stigma with paint brushes, and the flower was then re-covered. To prevent cross-pollination, we washed the paint brushes with distilled water and ethanol after pollinating each flower, and then dried the brushes before

TABLE 1. Timetable of anthesis in flowers of *Polaskia chichife* and percentage of flowers per population type showing particular schedules.

Season	Time of day	Hours open	Percentage of flowers		
			Wild	Managed in situ	Cultivated
Winter	1000–1200	26	10.00	0.00	6.67
	1000–1300	27	10.00	16.67	16.67
	1100–1400	27	10.00	16.67	46.67
	1100–1600	29	33.33	40.00	3.33
	1100–1700	30	30.00	20.00	23.33
	1100–1800	31	6.66	6.66	3.33
Mean \pm 1 SE		28.41 \pm 0.16	28.73 \pm 0.28	28.66 \pm 0.24	27.83 \pm 0.27
Spring	0730–2130	14	10.00	35.00	20.00
	0730–2230	15	0.00	5.00	0.00
	0830–2130	13	35.00	25.00	5.00
	0830–2230	14	0.00	0.00	25.00
	1000–2230	12.5	40.00	5.00	10.00
	1000–2330	13.5	0.00	30.00	0.00
	1100–2230	11.5	10.00	0.00	0.00
	1100–2330	12.5	0.00	0.00	30.00
	1200–2230	10.5	5.00	0.00	0.00
	1200–2330	11.5	0.00	0.00	10.00
Mean \pm 1 SE		13.12 \pm 0.10	12.68 \pm 1.51	12.58 \pm 0.66	13.10 \pm 0.88

reusing them. This treatment tested the hypothesis that failure of self-pollination is not due to physical factors related to flowers structure or to temporal factors related to behavior. A total of 24 individuals and 114 flowers from wild populations, 30 and 108 from managed in situ populations, and 29 and 118 from cultivated populations was sampled.

3. *Manual cross-pollination*—Pollen from the flowers of one individual was manually deposited on stigmas of a different individual, and the flowers were then re-covered with exclusion bags. A total of 14 individuals and 72 flowers from wild populations, 16 and 79 from managed in situ populations, and 15 and 73 from cultivated populations was sampled.

4. *Natural pollination (control)*—Flower buds just before anthesis were labeled and maintained without exclusion bags until the fruits began to grow. At that point, the flowers were covered to protect the fruits. A total of 29 individuals and 457 flowers from wild populations, 30 and 609 from managed in situ populations, and 26 and 599 from cultivated populations was sampled.

For each pollination treatment, fruit and seed set were determined, and differences between treatments per population type were analyzed with one-way analyses of variance (ANOVA). Seed viability was determined by germinating seeds resulting from the experiments described below.

Diurnal vs. nocturnal pollination—For each of the following treatments, a total of 20 flower buds from ten individuals in the wild population of San Luis Atolotitlán were bagged in winter (20–21 January) and spring (27 March). The bags were removed as indicated for each treatment, and then the flowers were re-covered for 1 mo, until the collection of successful or aborted fruits.

1. *Complete diurnal pollination*—This treatment covered the entire daylight period during which flowers were open. In winter, exclusion bags were removed from the flowers from 0800 to 1830 on the first day, the flowers were re covered at night, and the bags were then removed from 0800 until the flowers closed on 21 January (1800). In spring, bags were removed from 0730 to 1930 on 27 March.

2. *Diurnal pollination only on the first day*—This treatment was used in the winter experiments to test whether complete diurnal pollination substantially increased the production of fruit. Exclusion bags were removed only during the first day of anthesis (from 0800 to 1830) and then the flowers were re-covered.

3. *Diurnal pollination only on the second day*—For the same purpose as treatment 2, and also in winter, exclusion bags were removed only during the daylight of the second day of anthesis (from 0800 to 1800).

4. *Nocturnal pollination*—Exclusion bags were removed from the flowers when it was dark. In winter, bags were removed from 1830 to 0730 of the following day, and in spring from 2000 to 0630, the flowers were then re-covered.

5. *Control*—Flower buds just before anthesis were labeled and were not covered with exclusion bags.

Phenology—Ten individuals in each of the wild, managed in situ, and cultivated study populations were observed for production of flowers and fruits throughout the reproductive season. Five principal branches per individual were randomly selected, and the number of flower buds, flowers in anthesis, immature fruits, and mature fruits was counted every 30 d.

Seed germination experiments—Seeds of three fruits per plant were obtained from 25, 27, and 30 individuals in wild, managed in situ, and cultivated populations, respectively, in San Luis Atolotitlán. A total of 30 randomly chosen seeds per plant were put on three layers of moist filter paper in plastic 9-cm-diameter petri dishes, which were arranged in a random design within a growth chamber. The chamber was set for alternating day/night temperatures (27/12°C) and 14/10 h photoperiod in order to simulate the temperature and light conditions of natural germination. The number of germinating seeds (emerging hypocotyls) was counted every 24 h, and the germination percentage calculated per day per population. The germination capacity (GC) (percentage of seeds that germinated at the end of the experiment), the median germination rate (R_{50}) (days required for 50% germination of the seeds), and the germination speed (R_{90}) (days required for 90% of the seeds to germinate, according to Thompson and El-Kassaby, 1993) were calculated. The GC data were transformed by arcsine, and R_{50} and R_{90} by $(1 - [1/x + 1])$, where x is the variable transformed, in order to normalize the calculated response variables and to achieve homogeneity of variances. The transformed data were analyzed by one-way ANOVA for testing differences between populations.

RESULTS

Floral biology—Flower anthesis of *P. chichife* in all the populations studied was predominantly diurnal, but during winter, flowers were open longer than during spring (Table 1). In winter, flowers were open 28.41 ± 0.16 h (mean \pm 1 SE, $N = 90$ flowers), ~ 12 h of which are at darkness, whereas in spring, flowers were open 13.12 ± 0.10 h ($N = 60$ flowers), only 2.82 ± 0.10 h ($N = 60$ flowers) of which were during the night. In the two seasons, flowers of the three populations studied were

TABLE 2. Timetable of the main events of anthesis in flowers of *Polaskia chichipe* during winter and spring and percentage of flowers participating.

Event	Winter		Spring	
	Time of day	Percentage of flowers	Time of day	Percentage of flowers
Start of opening	0900	73	0900	58
Flowers completely open	1200	100	1200–1300	100
Pollen release	1300	80	1300–1330	85
Maximum turgidity of stigma	1330	80	1330	85
Start of closing	1400 ^a	73	1700	80
Flowers completely closed	1700 ^a	93	2230	90

^a Hour of the day after the start of anthesis.

open in overlapping intervals. In most of the flowers observed, the outermost layer of tepals started to separate at ~0900, pollen release occurred at ~1300, and maximum turgidity of stigmas was reached at 1330 (Table 2). During winter, most flowers stayed open, with pollen available and stigmas turgid, until ~1400 of the following day, when closing started to finish at ~1700. In spring, most flowers began to close at ~1700 and were closed at 2230 of the same day that anthesis started. At the beginning of anthesis, traces of nectar were perceived, and the maximum production was between ~1300 and ~1700, coinciding with the maximum turgidity of stigma (Fig. 2).

Flower visitors—During spring, we recorded at least 16 species of insects and birds visiting flowers of *P. chichipe*, whereas in winter, we observed only seven animal species (Tables 3 and 4). Flowers in winter had a significantly higher frequency of visits than those in spring. In winter, the most frequent diurnal visitor was *Xylocopa mexicanorum* Cockerell, which visited flowers from 1000 to 1700, but more intensely between 1200 and 1500 of both the first and second days of anthesis. The next most frequent visitors were the hummingbirds *Amazilia violiceps* Gould, *Cyanthus sordidus* Gould, and *C. latirostris* Swainson, which visited flowers between 1100 and 1900. At night, the visits of a hawk moth species (Lepidoptera, Sphingidae) were also significant. In spring, more species of bees were present than in winter, and *Apis mellifera* L., *X. mexicanorum*, and *Plabeia mexicana* Ayala along with the hummingbird *Amazilia violiceps* were the most frequent visitors to *P. chichipe* flowers. At night, the hawk moth also visited flowers, but not often because the flowers were open for significantly shorter periods than in winter. During spring, *A. mellifera* had the highest frequency of visits, starting at ~0900 and continuing the whole day. The honey bees were most active between ~1000 and ~1200, although they also visited frequently between ~1300 and ~1600. *Xylocopa mexicanorum* was active between ~0900 and ~1700, with the highest activity between ~1400 and ~1600. The bumble bee *Bombus pensylvanicus pensylvanicus* De Geer visited flowers only between ~1000 and ~1100, with relatively low frequency. The meliponinae bees *Plabeia mexicana* and *P. frontalis* Friese frequently visited flowers between ~1200 and ~1300 and again between ~1500 and ~1600.

The bees *X. mexicanorum*, *A. mellifera*, and *B. pensylvanicus pensylvanicus* loaded abundant pollen on their bodies, especially on their legs and abdomen. They all moved pollen among flowers from one or several individuals, invariably touching the stigmas. The meliponinae bees loaded less pollen than the other bees, but generally got into the flowers

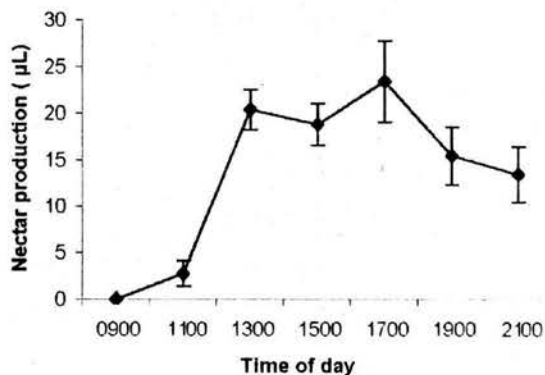


Fig. 2. Mean nectar production by flowers of *Polaskia chichipe* throughout anthesis (means \pm 1 SE).

through the space between the stamens and the style, a passage that has contact with both the stigma and the anthers.

The hummingbird *A. violiceps* visited a considerable number of flowers from different individuals located >1 km apart. Although the birds' beaks were longer than the flower tubes of *P. chichipe* (4.5 ± 0.17 cm [mean \pm 1 SE] and 1.83 ± 0.11 cm, respectively), hummingbirds commonly touch both anthers and stigmas with their beaks and faces during their visits. Sampling of pollen on the hummingbird visitors detected chichipe pollen on their heads and throats (María del Coro Arizmendi, Facultad de Estudios Superiores, Iztacala, Universidad Nacional Autónoma de México, México, unpublished data).

Black ants visited constantly throughout the day, destroying entire parts of *P. chichipe* flowers and buds. Beetles visited flowers during the day and part of the night, although at relatively low frequency. These insects were small (2.7 ± 0.23 mm [mean \pm 1 SE]) and entered the flowers through lateral spaces between the tepals, staying close to the nectarous chamber at the base of the reproductive structures without touching anthers and stigma.

Among nocturnal visitors were unidentified moths, hemipterans, and *Atta mexicana* L. ants. The most frequent nocturnal visitors during both winter and spring were hawk moths, which reached nectar with their proboscises, eventually touching anthers and stigmas. Visits of hemipterans and ants occurred in spring between ~2100 and ~2200 and between ~2000 and ~2200, respectively, at a relatively low frequency. Hemipterans stayed mainly on the tepals, not penetrating into the flower. *Atta mexicana* ants were predatory on flowers and buds.

Breeding system—Treatments for testing natural pollination were in all cases >80% successful in yielding fruit, whereas treatments entailing manual cross-pollination were 37.5–49.37% successful (Table 5). Nonmanipulated self-pollination was successful in ~45–46% of the individuals sampled in managed in situ and cultivated populations, but only in ~19 of individuals sampled in wild populations. This treatment was ~22–27% successful in flowers sampled in managed in situ and cultivated populations, but only ~12% successful in the wild. Manual self-pollination was also less successful in the wild (~12% of flowers, ~17% of individuals) than in the managed in situ (~24% of flowers, ~44% of individuals) and cultivated (~27% of flowers, ~62% of individuals) populations (Table 5). Fruits produced by self-pollination reached

TABLE 3. Frequency of visits to *Polaskia chichife* flowers during (A) the start of anthesis in winter and (B) the day after the start of anthesis in winter ($N = 45$ flowers, values are means ± 1 SE).

Visitor	No. visits per hour of the day													
	0900-1000	1000-1100	1100-1200	1200-1300	1300-1400	1400-1500	1500-1600	1600-1700	1700-1800	1800-1900	2300-2400	2400-0100	0200-0300	
A) Hymenoptera														
Black ant	0.2 \pm 0.20	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Xylocopa</i> sp.	0	0.4 \pm 0.25	0.6 \pm 0.25	0.6 \pm 0.25	0.2 \pm 0.20	0	0	0	0	0	0	0	0	0
<i>Xylocopa mexicana</i> - <i>rum</i>	0	1.6 \pm 0.25	3.8 \pm 0.20	6.4 \pm 0.51	9.2 \pm 0.38	6.2 \pm 0.38	0.6 \pm 0.25	0.8 \pm 0.20	0	0	0	0	0	0
Lepidoptera (Sphingidae)														
Hawk moth	0	0	0	0	0	0	0	0	0	0	1.2 \pm 0.38	1.2 \pm 0.38	0.4 \pm 0.25	0
Diptera														
<i>Antrax</i> sp.	0	0	0	0	0.2 \pm 0.20	0	0	0	0	0	0	0	0	0
Aves (Trochilidae)														
<i>Amazilia violiceps</i>	0	0	2.0 \pm 0.0	0.4 \pm 0.25	1.2 \pm 0.38	0	0	0	1.0 \pm 0.0	0	0	0	0	0
<i>Cyananthus sordidus</i>	0	0	0	0	0	1.0 \pm 0.0	0.2 \pm 0.20	0	1.0 \pm 0.0	1.0 \pm 0.0	0	0	0	0
B) Hymenoptera														
Black ant	0.4 \pm 0.40	0.6 \pm 0.15	0	0	0	0	0	0	0	0	0	0	0	0
<i>Xylocopa</i> sp.	0	0	0.5 \pm 0.26	0.33 \pm 0.26	0	0	0	0	0	0	0	0	0	0
<i>Xylocopa mexicana</i> - <i>rum</i>	0	0.8 \pm 0.20	3.0 \pm 0.82	4.0 \pm 0.89	12.0 \pm 1.89	8.5 \pm 1.58	1.0 \pm 0.63	1.5 \pm 0.32	0	0	0	0	0	0
Aves (Trochilidae)														
<i>Amazilia violiceps</i>	1.0 \pm 0.32	0	2.0 \pm 0.0	0.33 \pm 0.26	1.0 \pm 0.00	0	0	0	1.5 \pm 0.32	1.5 \pm 0.32	0	0	0	0
<i>Cyananthus sordidus</i>	0	0	0	0	0	2.0 \pm 0.0	1.0 \pm 0.00	0	1.0 \pm 0.00	1.0 \pm 0.00	0	0	0	0

TABLE 4. Frequency of visits of *Polaskia chichipe* flowers during anthesis in spring ($N = 45$ flowers, values are means ± 1 SE).

Visitor	No. visits per hour of the day												
	0900-1000	1000-1100	1100-1200	1200-1300	1300-1400	1400-1500	1500-1600	1600-1700	1700-1800	1800-1900	1900-2000	2000-2100	2100-2200
Hymenoptera													
<i>Apis mellifera</i>	0.6 \pm 0.3	2.0 \pm 0.6	1.6 \pm 0.6	0.6 \pm 0.3	1.0 \pm 0.5	0.2 \pm 0.2	1.0 \pm 0.3	0	0.2 \pm 0.2	0	0.2 \pm 0.2	0	0
<i>Xylocopa mexicana-rum</i>	0.4 \pm 0.3	0.6 \pm 0.4	0	0.8 \pm 0.2	0.6 \pm 0.3	1.8 \pm 0.4	1.6 \pm 0.3	0.8 \pm 0.2	0	0	0	0	0
<i>Bombus pensylvanicus</i>	0	0.4 \pm 0.3	0	0	0	0	0	0	0	0	0	0	0
<i>Platbeia mexicana</i>	0	0	0	2 \pm 1.1	0	0	0.8 \pm 0.4	0	0	0	0	0	0
<i>P. frontalis</i>	0	0	0	0.4 \pm 0.3	0.2 \pm 0.2	0	0	0	0	0	0	0	0
Black ants	0.4 \pm 0.3	0.2 \pm 0.2	0	0	0.2 \pm 0.2	0.6 \pm 0.3	0.6 \pm 0.4	0	0.4 \pm 0.3	0	0	0.2 \pm 0.2	0
<i>Atta mexicana</i>	0	0	0	0	0	0	0	0	0	0	0	0.4 \pm 0.4	0.4 \pm 0.3
<i>Polistes mexicanus</i>	0	0	0.2 \pm 0.2	0	0	0.2 \pm 0.2	0	0	0	0	0	0	0
<i>Brachygastra mellifica</i>	0	0	0	0	0.4 \pm 0.3	0	0	0	0	0	0	0	0
Diptera (Bombyliidae)													
<i>Antrax</i> sp.	0	0	0	0.2 \pm 0.2	0.2 \pm 0.2	0	0	0	0	0	0	0	0
<i>Exoprosopa</i> sp.	0	0	0	0	0	0.2 \pm 0.2	0.4 \pm 0.3	0	0	0	0	0	0
Coleoptera													
Beetle	0	0	0	0.4 \pm 0.4	0	0	0	0	0	0	0	0	0.4 \pm 0.4
Hemiptera													
Aphididae	0	0	0	0	0	0	0	0	0	0	0	0	0.2 \pm 0.2
Lepidoptera (Sphingidae)													
Hawk moth	0	0	0	0	0	0	0	0	0	0	0	0	0.2 \pm 0.2
Aves (Trochilidae)													
<i>Anazilia violiceps</i>	0.8 \pm 0.2	0	0	0	0	0	0.4 \pm 0.3	0	0	0.8 \pm 0.2	0.4 \pm 0.3	0	0
<i>Cyananthus latirostris</i>	0	0	0	0	0.4 \pm 0.3	0	0	0	0.4 \pm 0.3	0.4 \pm 0.3	0	0	0
<i>C. sordidus</i>	0	0	0	0	0	0.4 \pm 0.4	0	0	0.4 \pm 0.4	0.4 \pm 0.4	0	0	0

TABLE 5. Percentage of successful individuals and flowers and seed production per fruit per pollination treatment (means \pm 1 SE) in cultivated, managed in situ, and wild populations of *Polaskia chichife* in two localities of the Tehuacan Valley. Mean numbers of seeds per fruit within and among treatments with the same letter did not differ at $P \leq 0.05$ after Student's *t* tests. $F = 136.51$, $df = 3, 410$, $P = 0.001$.

Population	Control			Nonmanipulated self-pollination			Manual self-pollination			Manual cross-pollination		
	Individuals	Flowers	Seeds ¹	Individuals	Flowers	Seeds ²	Individuals	Flowers	Seeds ³	Individuals	Flowers	Seeds ⁴
Wild N	93.10 29	85.34 457	305.45 \pm 10.89 ^B	19.35 31	11.80 356	24.65 \pm 4.63 ^E	16.66 24	12.28 114	13.44 \pm 30.16 ^F	57.14 14	37.50 72	309.04 \pm 6.37 ^{DE}
Managed in situ N	100.00 30	82.43 609	326.19 \pm 10.89 ^{AB}	46.42 28	27.00 352	38.35 \pm 6.73 ^E	43.33 30	24.07 108	97.23 \pm 21.94 ^{CD}	81.25 16	49.37 79	320.35 \pm 6.07 ^E
Cultivated N	100.00 26	84.14 599	338.91 \pm 10.80 ^A	44.83 29	22.22 630	28.73 \pm 5.36 ^E	62.07 29	27.12 118	102.52 \pm 18.09 ^{CD}	86.66 15	41.10 73	337.63 \pm 6.32 ^C

TABLE 6. Percentage of successful fruits produced in experiments on diurnal vs. nocturnal pollination of *Polaskia chichife* ($N = 20$ flowers per treatment).

Treatment	Percentage of successful fruits	
	Winter	Spring
Diurnal (complete)	40	100
Diurnal (only first day)	55	—
Diurnal (only second day)	35	—
Nocturnal	15	0
Control	60	100

maturity but seed production was significantly lower than in fruits resulting from both control and cross-pollination treatments (Table 5). Seedless fruits, seemingly parthenocarpic and significantly smaller than those with seeds, were recorded in 8.2% of the nonmanipulated self-pollinated plants.

Diurnal vs. nocturnal pollination—In diurnal experiments, fruit set was generally higher during spring than winter (Table 6). During winter, diurnal pollination was successful in all treatments, although the fruit set in the treatment that involved diurnal pollination only the second day was lower. It is significant that during this season nocturnal pollination was successful, although production was relatively low (15%). During spring, fruit production was successful in all treatments testing diurnal pollination, and all fruits aborted in treatments testing nocturnal pollination.

Crosses among populations—Crosses among population were highly successful when the pollen was from populations under the same type of management (Table 7). However, independent of the source of pollen, seed production was highest in fruits from cultivated populations, less in the managed in situ populations, and lowest in the wild populations. The lowest seed production occurred in crosses between wild and cultivated individuals and between wild and managed in situ individuals (Table 7).

Phenology—Production of flower buds started during the third week of January, occurring synchronically in the three types of populations studied (Fig. 3). Production of flowers in anthesis was recorded from January (in the three population types) to March in the wild populations, to April in the managed in situ populations, and to May in the cultivated populations. Figure 4 illustrates overlaps of blooming season in the three population types, as well as variations in flowering peak (February in the wild populations, March in the managed in situ populations, and April in the cultivated populations). Fruits started to ripen in March in the managed in situ populations, whereas in the wild and cultivated populations, this occurred in April. The reproductive season ended in June in the wild populations, in July in the managed in situ populations, and in August in the cultivated populations.

Seed germination experiments—Germination of seeds from all populations started on day 7 of the experiment, but by day 9, a significantly higher percentage of seeds from both cultivated and managed in situ populations started to germinate compared with seeds from the wild population (Fig. 5). After 34 d, germination percentage of seeds from the managed in situ and cultivated populations was 77.2% and 76.3%, respec-

TABLE 7. Results of crosses among cultivated, managed in situ, and wild populations of *Polaskia chichipe*. Mean numbers of seeds per fruit in crosses with the same letter did not differ at $P \leq 0.05$ after Student's t tests. $F = 2.96$, $df = 2, 62$, $P = 0.008$.

Population	Pollen source								
	Cultivated			Managed in situ			Wild		
	S_i/T_i	F_i/F_i	$N_i \pm 1 SE$	S_i/T_i	F_i/F_i	$N_i \pm 1 SE$	S_i/T_i	F_i/F_i	$N_i \pm 1 SE$
Cultivated	8/14	14/27	128.9 \pm 20 ^A	4/11	8/22	164.3 \pm 29 ^A	5/12	8/24	37.4 \pm 27 ^{AB}
Managed	8/15	13/29	61.4 \pm 24 ^B	11/14	17/26	140.0 \pm 50 ^B	8/15	9/24	52.8 \pm 24 ^B
Wild	3/13	4/26	36.3 \pm 41 ^B	2/11	2/19	48.3 \pm 20 ^{AB}	8/9	21/27	71.3 \pm 41 ^B

S_i = number of successful individuals, T_i = total number of individuals sampled, F_i = number of fruits, F_i = number of flowers sampled, N_i = mean number of seeds produced per fruit.

tively, significantly higher than the 60.8% in the wild population ($F = 4.03$, $P = 0.021$; Fig. 5). The speed of seed germination in both cultivated and managed in situ populations ($R = 0.65$, $R_{50} = 0.08$, and $R_{50'} = 0.1$) was significantly faster than in the wild population ($R = 0.46$, $R_{50} = 0.06$ and $R_{50'} = 0.08$; $F = 13.02$, 5.98, and 6.33, respectively; $P < 0.005$).

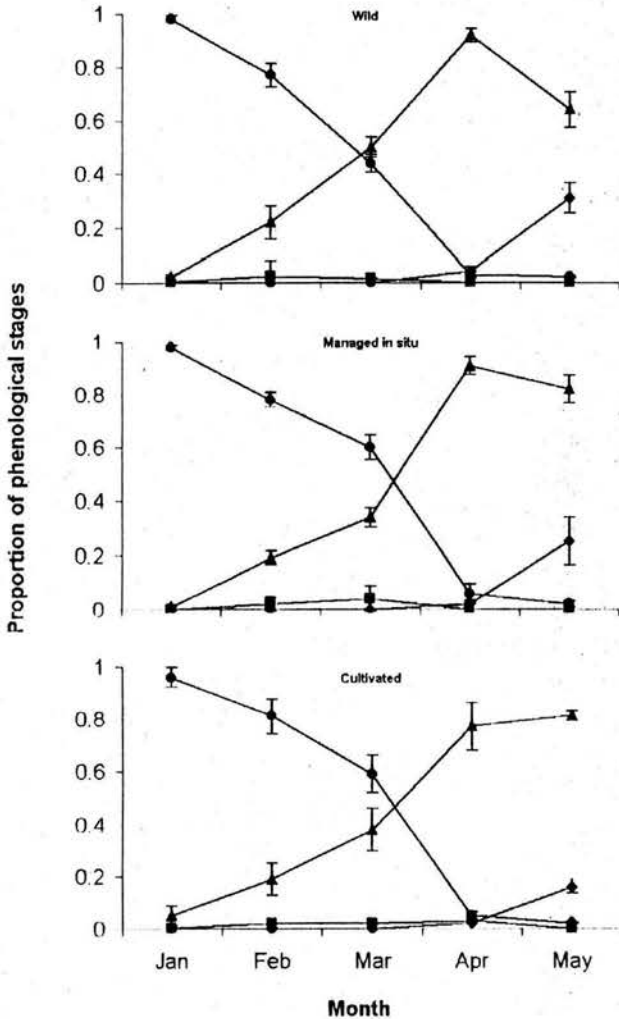


Fig. 3. Phenology of *Polaskia chichipe* in wild, managed in situ, and cultivated populations: circles represent flower buds, squares represent flowers in anthesis, triangles represent immature fruits, and diamonds represent mature fruits (means $\pm 1 SE$).

Seeds produced by self-pollination were viable without significant differences in germination parameters compared with seeds produced by cross-pollination (Table 8). However, germination percentage of seeds produced in both self-pollination and cross-pollination treatments was significantly lower (19.2% and 18.9%, respectively) than that of seeds produced in the control treatments (70%). The nonmanipulated self-pollination treatments yielded seeds with $14 \pm 0.03\%$ germination (mean $\pm 1 SE$, $N = 62$ fruits), whereas germination percentage of seeds from manual self-pollination treatments was $24 \pm 0.04\%$ ($N = 35$ fruits) and in seeds from cross-pollination treatments was $18.9 \pm 0.34\%$ ($N = 58$ fruits). Germination percentage of seeds produced by the different crosses (Table 9) was not significantly different in any of the cases.

DISCUSSION

Anthesis in *Polaskia chichipe* was predominantly diurnal (flowers open for ~ 16 h during the day in winter and ~ 10 h in spring). Periods of higher nectar production (~ 1300 – 1700) and frequency of visits to flowers (~ 1000 – 1700) notably coincided with the time when pollen started to be released and stigma lobes were turgid. This information suggests that pollination of *P. chichipe* is conducted mainly by diurnal visitors. Among them, *Xylocopa mexicanorum* was consistently a frequent visitor throughout the reproductive season and visited *P. chichipe* more often than the other bee species. *Apis mellifera*, *Plabeia mexicana*, and *P. frontalis* were also frequent visitors in spring. All these bees invariably touched anthers and stigmas during their visits and loaded significant amounts of pollen, especially *X. mexicanorum* and *A. mellifera*. Foraging activity of all these bees entailed short flights to flowers of one or several individuals. However, the availability of nectar from a given individual was variable throughout the day.

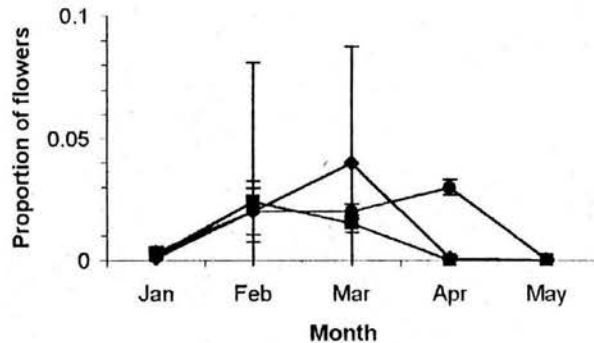


Fig. 4. Floral phenology in wild (squares), managed in situ (diamonds), and cultivated (circles) populations of *Polaskia chichipe* (means $\pm 1 SE$).

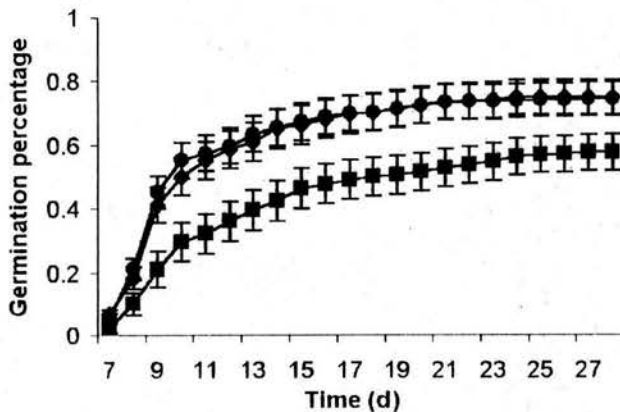


Fig. 5. Germination percentage and rate of seeds from individuals of wild (squares), managed in situ (diamonds), and cultivated (circles) populations of *Polaskia chichipe* (means \pm 1 SE).

The variability apparently increased visits to flowers of different plants, which in turn appeared to favor pollen exchange.

The hummingbirds were active during the period when pollination of *P. chichipe* apparently occurred, and they appeared to touch anthers and stigmas during their visits; thus, they could have a role in pollination of chichipe. Experiments that exclude the participation of bees and other flower visitors might confirm and measure the effectiveness of these birds as pollinators.

The bumble bee *Bombus pensylvanicus pensylvanicus* visited flowers of *P. chichipe* with low frequency and before pollen release and stigma maximum turgidity occurred. It is unlikely that this species is a pollinator of *P. chichipe*. It is also unlikely that ants, which prey on *P. chichipe* flowers, and beetles, which do not touch the anthers and style, have any role in pollination.

Anthesis was also partly nocturnal (flowers open for \sim 12 h during the night in winter and \sim 3 h in spring). According to our experiments, nocturnal visitors were effective pollinators only during winter. Nocturnal visits by hawk moths were frequent during winter, when flowers were open throughout the night, but their role as pollinators seemed to be irrelevant in spring.

The seemingly most important pollinators of *P. chichipe* were the same in the wild, managed in situ, and cultivated study populations. These populations were separated at most by 5.5 km. The distance that most of the bees visiting *P. chichipe* are able to fly has not been documented, but according to Metcalf and Flint (1974), *A. mellifera* may fly within a radius of nearly 2.5 km, indicating the possible movement of pollen at least between the wild and managed in situ populations (1.5 km apart). However, the populations studied were not discrete patches in the landscape. Groups of *P. chichipe* and of other columnar cacti (*Polaskia chende* [Gosselin] A. Gibson & K. Horak, *Escontria chiotilla* [F. A. C. Weber] Rose, and *Myrtillocactus schenkii* [J. Purpus] Backeberg) visited by the same bee species occurred between the study populations, sometimes creating continuous bridges of plants and thereby allowing pollen exchange between populations. Hummingbirds may cover distances $>$ 5.5 km/d (Arizmendi, 2001). Therefore, if these birds pollinate chichipe, that would increase the probability of pollen exchange between populations. All

TABLE 8. Germination percentage (means \pm 1 SE) of seeds produced by *Polaskia chichipe* per pollination treatment. Mean percentage of germinating seeds from fruits in pollination treatments with the same letter did not differ at $P \leq 0.05$ after Student's *t* tests. $F = 20.28$, $df = 2, 136$, $P = 0.0001$.

Pollination treatment	Fruits (N)	Germination percentage
Cultivated CP	19	0.177 \pm 0.060 ^{AB}
Cultivated NMSP	18	0.143 \pm 0.061 ^{AB}
Cultivated MSP	20	0.348 \pm 0.058 ^C
Cultivated control	30	0.76 \pm 0.050 ^E
Managed CP	28	0.208 \pm 0.049 ^{AB}
Managed NMSP	14	0.187 \pm 0.069 ^{BC}
Managed MSP	12	0.328 \pm 0.075 ^F
Managed control	27	0.771 \pm 0.052 ^{AB}
Wild CP	11	0.185 \pm 0.078 ^A
Wild NMSP	30	0.105 \pm 0.047 ^{ABC}
Wild MSP	3	0.046 \pm 0.150 ^B
Wild control	25	0.608 \pm 0.054
Total CP	58	0.189 \pm 0.034
Total NMSP	62	0.144 \pm 0.033
Total MSP	35	0.240 \pm 0.044
Total control	82	0.700 \pm 0.051

Note: CP = cross-pollination, NMSP = nonmanipulated self-pollination, MSP = manual self-pollination.

these aspects make the existence of spatial reproductive barriers unlikely.

Experiments on the breeding systems revealed that manual crosses were generally more successful than self-pollination. Self-pollination was generally more successful in managed in situ populations (\sim 43–46% individuals, \sim 24–27% flowers) and cultivated populations (\sim 45–62% individuals, \sim 22–27% flowers) than in the wild (\sim 17–19% individuals, \sim 12% flowers). Therefore, self-incompatibility appears to have been favored by human manipulation of populations. Fruits resulting from self-pollination treatments generally produced significantly fewer seeds than those from natural and manual cross-pollination treatments and had reproductive disadvantages compared with fruits from cross-pollination. However, because self-compatibility allows the possibility of maintaining fruit production during periods of scarcity of pollinators or their absence, individuals with this attribute would be relatively more productive and thus favored by artificial selection, by their sparing and enhancement by management in situ, and by their cultivation.

Patterns of fruit set from crosses between population types show levels of reproductive affinity in relation to the type of population management. In general, each population showed higher reproductive affinity when crossed with the other population under the same management regimen. The least suc-

TABLE 9. Germination percentage (means \pm 1 SE) of seeds produced from crosses among populations of *Polaskia chichipe*.

Population, pollen source	Fruits (N)	Germination percentage
Cultivated, cultivated	11	0.173 \pm 0.085
Cultivated, managed	5	0.193 \pm 0.131
Cultivated, wild	3	0.167 \pm 0.169
Managed, cultivated	11	0.286 \pm 0.085
Managed, managed	10	0.226 \pm 0.093
Managed, wild	7	0.059 \pm 0.110
Wild, cultivated	2	0.233 \pm 0.207
Wild, managed	2	0.033 \pm 0.207
Wild, wild	7	0.214 \pm 0.111

successful crosses were between wild and cultivated populations, and crosses of managed in situ populations with wild and cultivated populations were intermediately successful. Although the nature of such affinity is as yet unknown, it represents a partial barrier to pollen exchange among populations under different management regimens.

Our phenological studies indicate that pollen can be exchanged among wild, managed in situ, and cultivated populations between January and March, when production of flowers overlap in the three population types. However, flowering peak varied in the three types: February in the wild populations, March in the managed in situ populations, and April in the cultivated populations. Such phenological differences mean that management increases the time *P. chichipe* fruits are available: fruit production in wild populations ends in June, whereas in the managed in situ populations it ends in July and in the cultivated populations in August. During the flowering peak, resources for pollinators are concentrated in a given area, and a significant proportion of the fruits produced from flowers opening then may therefore contain seeds with genes from the same population. In other words, although pollen exchange between populations is possible, during flowering peaks it may be more frequent among individuals within the same population. In addition, pollen flow between at least the wild and cultivated populations is interrupted from April to May when the wild population stops producing flowers. These features, together with self-pollination and differential affinity to pollen exchange among populations under different management regimens, may create partial barriers to pollen exchange between populations under different types of management, especially between wild and cultivated populations. A. Carmona and A. Casas (unpublished data) found significant differences in fruit and seed size among wild, managed in situ, and cultivated populations (and especially between wild and cultivated populations), which apparently are the result of artificial selection for larger fruits. But maintenance of morphological differentiation is seemingly favored by the partial barriers arising from differences in pollen affinity, blooming time, and breeding system.

The germination experiments revealed a relatively high total germination percentage (~76–77%) of seeds from managed in situ and cultivated populations compared with the percentage (60%) recorded in the wild. The germination rate of seeds from managed in situ and cultivated populations was significantly higher than that of seeds from wild individuals. This feature could be related with seed size. A. Carmona and A. Casas (unpublished data) found that fruits and seeds were significantly larger and fruits had more seeds in managed and cultivated populations than in wild individuals. These authors consider that fruit size is directly related with seed size and number and that these latter characters could have resulted from artificial selection for larger fruits. In turn, the differences observed in germination rate could be a consequence of larger seeds. Human manipulation could have thus favored, indirectly, faster seed germination in *P. chichipe*, as it has in other cultivated columnar cacti (Rojas-Archiga, Casas, and Vázquez-Yanes, 2001).

In sum, research to date has shown that wild populations of *P. chichipe* significantly differ in morphology, especially in fruit and seed size, as well as in breeding system and seed germination patterns, compared with both managed in situ and

cultivated populations. All these differences are associated with human management and are therefore an apparent consequence of artificial selection.

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GENETIC VARIATION AND EVOLUTION OF *POLASKIA CHICHIPE*
(CACTACEAE) UNDER DOMESTICATION IN THE TEHUACAN VALLEY,
CENTRAL MEXICO.

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Abstract. - *Polaskia chichipe* is a columnar cactus under artificial selection for its edible fruits in Central Mexico. Our study explored the effect of human manipulation on levels and distribution of genetic variation in wild, silvicultural managed and cultivated sympatric populations. Total genetic variation, estimated in nine populations with five microsatellite loci, was $H_T = 0.658 \pm 0.026$ s.e., which was mainly distributed within populations ($F_{IS} = 0.126$) with low differentiation among them ($F_{ST} = 0.015$). Fixation index (F_{IS}) in all populations was positive, indicating a deficit of heterozygous individuals with respect to Hardy-Weinberg expectations. When populations were pooled by management type, the highest expected heterozygosity ($H_E = 0.631 \pm 0.031$ s.e.) and the lowest fixation index ($F_{IS} = 0.07$) were observed in wild populations, followed by cultivated populations ($H_E = 0.56 \pm 0.03$ s.e., $F_{IS} = 0.14$), whereas the lowest variation was found in silvicultural managed populations ($H_E = 0.51 \pm 0.05$ s.e., $F_{IS} = 0.17$). Low but significant differentiation among populations under different management types ($F_{ST} 0.005$, $P < 0.04$) was observed. A pattern of migration among neighboring populations, suggested from isolation by distance ($r^2 = 0.314$, $P < 0.01$) may have contributed to homogenizing populations and counteracting the effects of artificial selection. *Polaskia chichipe*, used and managed for long time, shows morphological differentiation, changes in breeding system and seed germination patterns associated with human management, with only slight genetic differences detected by neutral markers.

Keywords: columnar cacti, crop evolution, dinucleotide repeats, domestication, genetic structure, genetic resources, microsatellites.

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The semiarid Tehuacán-Cuicatlán Valley, Central Mexico has been identified as a key location for the origins of agriculture and plant domestication in the New World (MacNeish 1967). Peoples of this region domesticated a number of plant species, including species of columnar cacti (Casas et al. 1999; 2002). Currently, people in the region gather fruit from 20 species of columnar cacti from wild populations. They also practice silvicultural management of wild populations of 12 species in situ that involves favoring individuals with useful phenotypes when natural vegetation is cleared for agriculture (Casas et al. 1999). Seven of these species are also cultivated in homegardens and terraces, mainly by vegetative propagation (Casas et al. 1999; 2001).

Both cultivation and silvicultural management may involve domestication processes (Casas et al. 1999), that can lead to divergence between wild and manipulated populations (De Candolle 1882; Darwin 1883; Zohary

1984; Harlan 1992). Plant domestication and human manipulation of populations occur at varying intensities and can result in a continuum of morphological, physiological and genetic differentiation between wild and partially or fully domesticated variants. The study of both incipient and advanced degrees of domestication contributes to understanding of how the process of evolution under artificial selection operates. It also allows for evaluation of the impact of the current traditional management on genetic diversity. Columnar cacti in the Tehuacan Valley offer ideal biological systems for studying ongoing domestication processes and genetic interactions among wild and domesticated populations. Fruits of these plant species are the primary targets of artificial selection and farmers select on phenotypic characters such as fruit size, pulp color and flavor, peel thickness and thorniness (Casas and Barbera 2002).

Polaskia chichipe (Glosselin) Backeberg is one of the columnar cacti under silvicultural management and cultivation in the Tehuacán Valley (Casas et al. 1999). Previous studies (Carmona and Casas in press) documented that artificial selection has determined morphological differentiation among populations associated with management type. Fruits from cultivated populations are on average significantly larger, with more pulp and more and larger seeds than those from silvicultural managed populations and, in turn, fruits and seeds from silvicultural managed populations are larger than those from unmanaged wild populations (Carmona and Casas in press). Furthermore, artificial selection has favored a higher frequency of self-pollinating individuals, larger season of fruit production, faster and higher percentage of germination in populations manipulated by humans (Otero-Arnaiz et al. 2003). Partial temporal reproductive isolation and self-pollination appears to have contributed in part to maintenance of morphological and physiological divergence among population types (Otero-Arnaiz et al. 2003).

Populations under silvicultural management and cultivation are derived from wild populations, and all coexist in the same geographic area. Artificial selection on manipulated populations of *P. chichipe* involves tolerating, enhancing in abundance or propagating desirable individuals while clearing non-desirable individuals from agricultural fields and pastures. Propagation of desirable individuals is conducted by sowing seeds and planting branch cuttings (Carmona and Casas in press). Considering these patterns of management we expected to find a reduction of genetic variation in populations manipulated by humans with respect to wild populations. Such reduction was expected to be stronger in silvicultural managed populations since artificial selection involves the removal of part of a population when clearing vegetation. However, in cultivated populations genetic diversity maybe higher because people may introduce new local variants and variants from other towns, as has been documented for *Stenocereus stellatus* by Casas et al. (2001).

Genetically structured populations of *P. chichipe* are expected because previous experiments with crosses among and within populations demonstrated that fruit production was more successful when pollen was from the same population. Additionally, flowering peaks in wild, silvicultural and cultivated populations occur with a difference of one month respectively, determining partial temporal isolation among these populations (Otero-Arnaiz et al. 2003).

Changes resulting from silvicultural management, in which some individuals are deliberately eliminated, could cause significant reductions in population size. This type of management has the potential to create

genetic bottlenecks in which effective population size (N_e) is reduced. This reduction can be estimated by microsatellite markers and novel statistical methods (Cornuet and Luikart 1996; Piry et al. 1999; Maudet et al. 2002). Populations that have experienced a recent reduction of their effective population size (between $2N_e$ and $4N_e$ generations, according to Piry et al. 1999) exhibit a correlative reduction of the allele numbers (A_0) and gene diversity (H_E , or HW heterozygosity) at polymorphic loci. The number of alleles is reduced faster than gene diversity, therefore, in a recently bottlenecked population the observed gene diversity is higher than the expected equilibrium gene diversity (H_{eq}) computed from the observed number of alleles (A_0), under the assumption of mutation-drift equilibrium (Cornuet and Luikart 1996). The degree of severity of the potential bottleneck created by silvicultural management type will depend upon the genetic variation existing in the stand of plants maintained and it will be less severe if supplementary sources of pollen or seeds significantly contribute to the regeneration of the population.

In other species of columnar cacti studied, genetic structure among populations, measured as G_{ST} , is usually low; the only exception being *Lophocoreus schotti*, which has a relatively high G_{ST} among populations from separated geographic regions in Sonora and Baja California, Mexico (Hamrick et al. 2002). *P. chichipe* is an endemic species restricted to a small region in areas of volcanic soils in the Tehuacan Valley and cultivated in homegardens of some villages of the region. The primary diurnal visitors to *P. chichipe* flowers can potentially travel distances further than between populations (0.4-9.4 km), *Apis mellifera* may fly within a radius of nearly 2.5 km (Metcalf and Flint 1974), and hummingbirds may cover distances > 5.5 km/d (Arizmendi 2001). However, the pattern of foraging activity is more frequently restricted to short distances (Otero-Arnaiz et al. 2003). We expected that pollen movement among populations was limited and that *P. chichipe* would have low inter-population genetic variation that could increase with geographic distance.

Given the known patterns of management, artificial selection, and reproductive biology of *P. chichipe*, as well as the patterns of genetic structure reported for other columnar cacti, with this study we explored the following topics:

- 1) The effects of management practices on genetic diversity of this cactus species. We expected that variation would be relatively high in wild populations, lower in silvicultural populations and intermediate in cultivated populations; with genetic differentiation among populations associated to management type.

- 2) The effect of migration in relation to geographic distance in the genetic structure of populations. We expected that if migration is occurring among

neighboring populations under different management, it could counteract the effects of artificial selection.

3) The frequency of rare alleles and clones in populations, hypothesizing that these could be more frequent in cultivated populations, where people may plant materials from distant sites.

4) The levels of inbreeding among populations under different management types. According to previous studies on reproductive biology in *P. chichipe* (Otero-Arnaiz et al. 2003); the level of inbreeding could be higher in manipulated populations than in wild populations.

This study used microsatellites to survey the genetic variation and structure of wild, silvicultural and cultivated populations of *P. chichipe*. The results are interpreted in the light of information derived from studies of morphological variation (Carmona and Casas in press) and reproductive biology (Otero-Arnaiz et al. 2003), for greater understanding of the evolutionary consequences of human management of *P. chichipe* populations.

MATERIALS AND METHODS

Populations, individuals and plant tissue sampled

A total of nine populations (three wild, three silvicultural managed, and three cultivated) were sampled within and around the villages of San Luis Atolotitlán, Caltepec, and Metzontla, in the State of Puebla, Mexico (Fig. 1, Table 1). Flower buds were collected from 30 individuals per population and maintained at -70°C until DNA extraction. A method recommended for DNA extraction of cactus (de la Cruz et al. 1997), modified for miniprep, was used.

Microsatellite development and genotyping

Five microsatellite loci were identified specifically for *P. chichipe*. Three of them were described previously (Otero-Arnaiz et al. in press), and two were

developed de novo following the same procedure (Table 1). PCR reactions were performed using 7.5µl reactions: 50-100ng DNA, 0.25mM each dNTP, 2.5mM MgCl₂, 0.5µM of each primer (fluorescent with either FAM, HEX or TET), 0.25 units of Taq DNA polymerase (Perkin Elmer), and 1x Taq buffer (Perkin Elmer), 1x Taq master buffer. The reactions were denatured at 94°C for 4 min, followed by 25 cycles of denaturing at 94°C for 1min, annealing at primer-specific temperatures (Table 1) for 1min, extension at 72°C for 2 min and a final extension at 72°C for 4 min, using a GenAmp 9700 thermocycler (Applied Biosystems). PCR products were mixed with formamide and ROX-500 size standard (Applied Biosystems). Samples were denatured 2 min at 95°C. Analysis of microsatellite loci was conducted using an ABI 377-96 DNA sequencer in GENESCAN mode to detect the labeled primers and internal size standard in a 4.5% denaturing polyacrylamide gel. Allele size and scoring was performed with GENOTYPER software (Applied Biosystems).

Genetic variation within and among populations and management types

Linkage disequilibrium was estimated between all pairs of loci using the program GENEPOP (Raymond and Rousset 1995), which performs a probability test using a Markov chain (dememorization 5000, batches 100, iterations per batch 500). For each population of *Polaskia chichipe*, genetic diversity was estimated by the common genetic parameters: observed alleles (A_0), observed heterozygosity (H_0) and expected heterozygosity (H_E) under the hypothesis of Hardy-Weinberg (HW) equilibrium (Nei, 1978).

Differences with respect to HW equilibrium were calculated by Wright's F_{IS} (Weir and Cockerham 1984) and heterozygote deficiency was tested using Markov chain randomization (dememorization 1000, batches 100, iterations per batch 1000) to estimate score test (U test) through GENEPOP (Raymond and Rousset 1995).

TABLE 1. *Polaskia chichipe* microsatellites, primer sequences, number of alleles (n), and range of allele size.

Primer name	Primer sequence (5' to 3')	n	Size	Fluorescence	Annealing temperature
Pchi9*	GTGGCCGAGAAAGAAGTTTG AAAGGCCCAAATCATAAGCA	6	208-218	6FAM	60°C
Pchi21*	CGTTTAGCCCCTCTTTCTCC GTTCCCAACTGACCGACAAC	8	120-134	HEX	60°C
Pchi54*	CCTTGAGCTTTGACATTGAGA GGAAGGTTTTTCATTGGATGAG	11	158-182	HEX	60°C
Pchi20	GTGGCCGAGAAAGAAGTTTG AAAGGCCCAAATCATAAGCA	9	230-256	6FAM	56°C
Pchi50	CCTGGCAAACCTCTGTTTA GTTCCCAACTGACCGACAAC	8	216-230	TET	56°C

*Primers designation as in Otero et al. (in press)

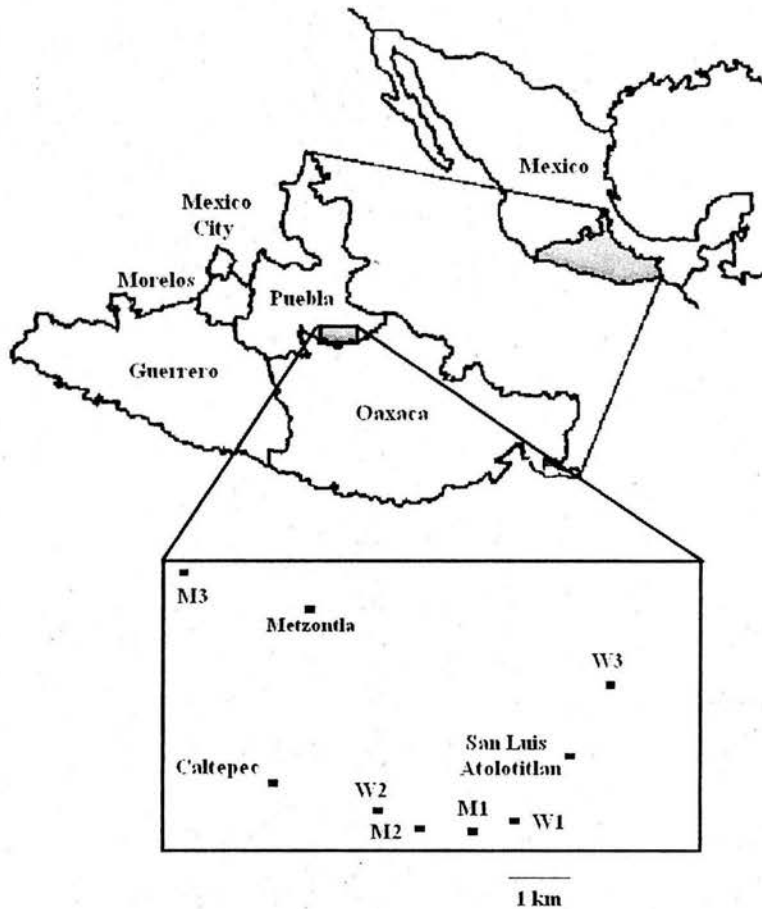


FIG. 1. Study area. The Tehuacán Valley. Sampled locations in the Municipality of Caltepec and Zapotitlan, Puebla, Mexico.

Differences in inbreeding coefficient (F_{IS}) among populations and management type were tested by Wilcoxon rank test performed with SIMSTAT ver 2.06 (Péladeau 1996). The number of exclusive alleles was determined by inspection of the allele distribution in populations.

The Wright's fixation indexes (Wright 1965) were estimated for all populations and pooling within and among management types using SPAGeDi 1.1 (Hardy and Vekemans 2002). These parameters are estimates of the correlations of genes within individuals relative to populations (F_{IS}) and of individuals and populations relative to all populations or the species (F_{IT} and F_{ST} , respectively). Additionally, an equivalent estimate of F_{ST} (R_{ST}) based in allele size differences, was calculated using the program RST22 (Goodman 1997). Since positive values of F_{IS} or inbreeding coefficient indicate deviations from HW equilibrium that can result by self pollination or mating among relatives, we expected more positive values in manipulated than in wild populations. Measures of genetic structure (F_{IS} , F_{IT} , F_{ST} and R_{ST}) were calculated independently for cultivated,

silvicultural and wild populations, considering every management type as a total population. Significance levels of the statistics were determined with 10,000 permutation tests.

There is not consensus yet on whether F_{ST} or R_{ST} based approaches are better for handling microsatellite data. Both have disadvantages: R_{ST} estimators overestimate differentiation if microsatellite frequently mutate by large insertions or deletions (Di Rienzo et al. 1994), on the other hand, F_{ST} estimators underestimate differentiation if mutation is largely stepwise (Slatkin 1995). We compared R_{ST} and F_{ST} values using a simple testing procedure proposed by Hardy et al. (2003) based on allele size randomizations under the null hypothesis of no contribution of stepwise mutation to genetic differentiation $R_{ST} = F_{ST}$. The test compare R_{ST} computed after allele size permutation (pR_{ST}), that depends solely on allele identity/nonidentity with the value computed before randomization (pR_{ST} should equal in expectation the F_{ST} computed on the same data). The probability that the null hypothesis holds is estimated as the proportion of pR_{ST} values larger than

the observed R_{ST} (one tailed test). Single locus and multilocus global F_{ST} (Weir and Cockerham 1984) and R_{ST} (Michalakis and Excoffier 1996) were estimated as well as pR_{ST} after 10,000 permutations with the program SPAGeDi (Hardy and Vekemans 2002).

Mating patterns

To estimate levels of selfed versus outcrossed offspring among maternal individuals, fruits of 7 to 14 (mean = 10.7) mother trees were sampled from a total of 6 populations (2 cultivated, 2 silvicultural managed and 2 wild) in April 2001. Seeds were germinated in January 2002. From each population, between 11 and 17 seedlings per mother tree per population (956 seedlings in total) were genotyped.

For each population, the mean outcrossing (t) and selfing ($s = 1-t$) rates were estimated, as well as the maternal inbreeding coefficient (f). A minimum estimate of the apparent selfing due to biparental inbreeding was calculated as the difference between single-locus selfing rate and multilocus selfing rate ($bi = tm-ts$) and the proportion of plants with exclusively outcrossing progeny (T). Expectation-maximization iteration was used to find maximum likelihood estimations to infer allele frequencies in the gene pool and the proportion of progeny that are the result of outcrossing using the program MLTR 2.4 (Ritland 2002). Standard errors for the outcrossing rates were calculated from 1000 bootstrap, using the family as the unit of resampling.

Relationship between geographic and genetic distance

Isolation by distance was tested using the method developed by Rousset (1997). Geographic distance among pairs of population was measured as the straight line distance between the estimated centers of populations. This method uses estimates of $F_{ST}/1 - F_{ST}$ for pairs of populations, and is based on the expected increase of genetic differentiation between pairs of populations according to geographic distance. Paired $F_{ST}/1 - F_{ST}$ estimations for the 9 populations were calculated with SPAGeDi 1.1 (Hardy and Vekemans 2002) and then a regression analysis of these estimates against the logarithm of the Euclidean distance was performed. The significance of isolation by distance was tested by a randomization process where populations were permuted among locations 10,000 times, which provides the null hypothesis of no correlation among geographic and genetic distances. P values were estimated as the proportion of this distribution falling above the observed regression slope.

In order to visualize the genetic relationship among populations, a matrix of genetic distances (Nei, 1972)

was calculated and a clustering dendrogram was constructed using the UPGMA method by TFGA (Miller 1997). Confidence of the tree constructed by the original data was estimated by 10,000 bootstraps with replacement over loci.

Reductions in effective population size

Detection of bottlenecks in populations was conducted using the two-phased model of mutation (TPM), as recommended by Pyrrri et al. (1999). Because few loci follow the Stepwise Mutation Model (SMM or strict single step mutations), tests for bottleneck were performed with 95% of SMM and 5% of multiple step mutation and a variance of 12 among multiple steps. A Wilcoxon sign-rank test was performed with Bottleneck software (Cornuet and Luikart 1996), which was used to determine if a population exhibits a significant number of loci with gene diversity excess.

RESULTS

Genetic variation within and between populations and management types

A total of 42 alleles were detected in the five microsatellite loci analyzed. Number of alleles per locus ranged from 6 to 11, averaging 8.4 alleles per locus. The least polymorphic locus was Pchi9, and the most polymorphic one was Pchi54 (Table 2). Out of the 264 individual plants sampled, a total of 260 different genotypes were identified, indicating that only about 2% of the individuals sampled could be clones derived from vegetative propagation, through planting branches as has been reported by local people (Carmona and Casas in press). One identical genotype was observed in two individuals of the silvicultural population M3, and this genotype was also observed in one individual from the wild population W1. Two other individuals of the cultivated population C3 and one of population C1 shared an identical genotype. Similarly, populations M2 and W2 shared two identical genotypes.

All five loci had one markedly common allele and one or more relatively rare alleles. At three loci the most common allele was the same in all nine populations. At Pchi21 and Pchi50 the most common allele was different in populations C1 and W3, respectively. Allele frequencies are available by request from the corresponding author.

Significant linkage disequilibrium ($P < 0.01$) was found between Pchi21 and Pchi50 in four of the nine populations studied. Linkage disequilibrium can be caused by several factors including physical linkage, and epistatic selection. These two loci have not been

TABLE 2. Geographic locations and genetic variation estimates nine *Polaskia chichipe* populations under different types of management in Tehuacan Valley, Mexico. Populations are numbered according to the territory of the municipality that they belong: 1. Atolotitlan, 2 Caltepec and 3 Mentzontla (Wild, silvicultural and cultivated populations abbreviated as W, S and C, respectively). Mean observed number of alleles per locus (A_O), mean observed and expected heterozygosities (H_O and H_E), with their se and a measure of heterozygote deficiency (F_{IS}) for the populations of *Polaskia chichipe* with different levels of management are shown.

Population	Latitude (N)	Longitude (W)	A_O	H_O	H_E	F_{IS}
W1	18°10'43.0"	97°26'38.4"	6.2±1.158	0.587±0.084	0.673±0.092	0.130**
W2	18°10'39.1"	97°27'21.8"	5.8±0.663	0.613±0.079	0.635±0.084	0.036
W3	18°11'54.3"	97°24'56.6"	5.8±0.735	0.693±0.062	0.726±0.069	0.048
S1	18°10'38.8"	97°26'49.9"	6.8±0.86	0.590±0.092	0.668±0.101	0.119*
S2	18°10'37.3"	97°27'6.4"	4.6±0.98	0.439±0.103	0.586±0.112	0.254**
S3	18°13'51.9"	97°29'42.3"	4.4±0.872	0.493±0.079	0.559±0.094	0.121*
C1	18°11'13.4"	97°25'13.0"	6.4±0.872	0.608±0.072	0.651±0.081	0.066
C2	18°10'37.3"	97°27'6.4"	6.2±0.735	0.587±0.051	0.674±0.065	0.131*
C3	18°13'19.8"	97°28'17.4"	5.2±0.583	0.488±0.075	0.631±0.074	0.229**
Wild				0.631±0.031	0.683±0.043	
Silvicult.				0.507±0.046	0.621±0.054	
Cultivated				0.560±0.030	0.660±0.039	

Exact test for heterozygote deficit using a Markov chain (dememorization 10000, batches 100, iteration per batch 1000) *P < 0.01, **P < 0.001

mapped in chromosomes of *P. chichipe*, but because the linkage disequilibrium was not present in all populations we could exclude physical linkage. However, we would expect a few significant tests for linkage disequilibrium by chance, considering that a large number of tests were conducted.

Genetic variation (H_E) across all populations varied from 0.559 ± 0.094 se in population M3 to 0.726 ± 0.069 s.e. in population W3 (Table 2). Population M1 with two unique alleles had the highest mean observed number of alleles per locus (6.8). Whereas, cultivated populations, each with a unique allele, had 5.2 to 6.4 alleles per locus. The average genetic variation within-populations (H_E) was higher in wild populations (0.685 ± 0.16 s.e.), than in cultivated populations (0.660 ± 0.132 s.e.), and the lowest occurred in silviculturally managed populations (0.621 ± 0.248 s.e.). Values of H_O in all populations were lower than H_E values, indicating

a deficiency of heterozygous individuals with respect to Hardy-Weinberg expectations. This deficit was significant in six populations, including two cultivated, all the populations under silvicultural management and one wild population (Table 2).

When populations under a similar type of management were analyzed together, inbreeding coefficients were significantly different from zero after a randomization test (U test) in all cases. The F_{IS} in silvicultural populations was higher than in cultivated populations, and the lowest F_{IS} was observed in wild populations (Table 3). Differentiation among populations was low but highly significant ($F_{ST} = 0.015$; $P < 0.001$) with a low, but significant, contribution due to differentiation among populations under different management type ($F_{ST} = 0.005$; $P < 0.04$). The strongest differentiation among populations under similar management type was found within silvicultural and

TABLE 3. Multilocus estimates of hierarchical F -statistics and their significance after 10,000 permutation tests for management type and for all populations of *Polaskia chichipe* studied in Tehuacan Valley, Mexico.

Management type	F_{IS}	F_{IT}	F_{ST}	R_{ST}
Wild	0.071**	0.080**	0.009*	0.001
Silvicultural	0.173***	0.190***	0.022**	0.0189*
Cultivated	0.142***	0.157***	0.017**	0.0183*
Among populations under different management	0.135***	0.140***	0.005*	
<i>Polaskia chichipe</i>	0.126***	0.140***	0.015***	0.009*

*P < 0.05, ** P < 0.01, ***P < 0.001

TABLE 4. Genetic differentiation and distances between *Polaskia chichipe* populations in Tehuacan Valley, Mexico. Above the diagonal, the differentiation estimation F_{ST} , below the diagonal, the R_{ST} computed using allele frequencies of 5 microsatellite markers.

	C1	M1	W1	C2	M2	W2	C3	M3	W3
C1		0.001	-0.001	0.011	0.004	-0.005	0.019	0.008	0.005
M1	-0.002		-0.004	0.021	0.000	0.008	0.039	0.038	0.016
W1	0.026	-0.002		0.003	0.008	0.003	0.027	0.023	0.011
C2	0.015	-0.001	-0.013		0.032	0.007	0.021	0.006	0.022
M2	0.012	-0.003	0.001	0.006		0.009	0.050	0.035	0.025
W2	-0.001	-0.012	0.004	-0.002	0.000		0.023	0.019	0.013
C3	0.032*	0.036*	0.029*	0.009	0.061*	0.030		0.017	0.024
M3	-0.003	0.021	0.043*	0.023	0.052	0.025	0.005		0.048
W3	0.002	-0.002	-0.008	-0.013	0.004	0.002	0.008	0.005	

cultivated populations ($F_{ST} = 0.021$ and 0.016 , respectively), wild populations were the least different among themselves ($F_{ST} = 0.009$, $P < 0.04$).

Differentiation based on allele sizes (R_{ST}) is shown in Table 4 for comparisons with other studies. However, F_{ST} seems to be a better estimate of genetic differentiation in *P. chichipe*, because permutation test did not detect any R_{ST} value significantly larger than pR_{ST} (Table 5).

TABLE 5. Differentiation among populations of *Polaskia chichipe*, estimated by global R_{ST} , mean pR_{ST} , and F_{ST} values per locus and for a multilocus average in Tehuacan Valley, Mexico.

	R_{ST}	pR_{ST}	F_{ST}
Multilocus	0.009 ^{NS}	0.016 (-0.002, 0.050)	0.015
Pchi21	0.023 ^{NS}	0.011 (-0.013, 0.054)	0.010
Pchi54	0.015 ^{NS}	0.032 (-0.008, 0.096)	0.035
Pchi9	0.027 ^{NS}	0.038 (0.007, 0.064)	0.042
Pchi50	0.007 ^{NS}	0.001 (-0.013, 0.015)	0.001
Pchi20	-0.001 ^{NS}	0.003 (-0.012, 0.028)	0.002

Mating patterns

Mating system parameters did not differ significantly among populations or management types (Table 5). Multilocus estimates of the proportion of outcrossing progeny (t_m) ranged from 0.82 to 0.98 and averaged 0.90 (± 0.09 s.e.). The fixation index of maternal plants (f) varied from zero to 0.06 and averaged 0.013 (± 0.06 s.e.), not significantly different to zero. Biparental inbreeding (bf) ranged from 0.005 to 0.102 and averaged 0.064 (± 0.07 s.e.).

On average, cultivated populations had the highest estimate of t_m (0.935 ± 0.06 s.e.) and silvicultural populations the lowest one (0.857 ± 0.08 s.e.). Cultivated populations also had the highest estimates of

fixation index of maternal plants and biparental inbreeding ($f = 0.034 \pm 0.07$ s.e. and $bf = 0.084 \pm 0.05$ s.e.). The proportion of individuals with exclusively outcrossed offspring ranged among 0.14 and 0.55. On average, cultivated populations had the lowest proportion of individuals without selfed offspring (0.26), whereas the wild population had the highest (0.40) and the silvicultural populations had an intermediate proportion (0.32).

Relationship between geographic and genetic distance

Genetically, the most similar populations were C1 and W2 ($F_{ST} = -0.005$, $R_{ST} = -0.001$) and the most different were M2 and C3 ($F_{ST} = 0.051$, $R_{ST} = 0.061$, Table 6). A positive correlation between genetic differentiation ($F_{ST} / 1 - F_{ST}$) and geographic distance in log scale was revealed by a randomization test. This correlation was statistically significant, with a significant $b = 0.0096$ after 10,000 permutations of locations among populations ($P \leq 0.007$, Fig. 2), indicating a restricted gene flow and a pattern of isolation by distance (Rousset 1997). There was a high sampling variance inherent to pairwise $F_{ST} / 1 - F_{ST}$ ratios. The geographic distance between populations explained only 31.4% of the total genetic variation. The slope of the regression was 0.009, indicating an indirect estimation of the neighborhood size of 104 individuals (from $b = 1 / 4N\pi\sigma^2$, see Rousset 1997). Regression coefficient of $F_{ST} / 1 - F_{ST}$ ratios was higher between pairs of populations under the same management type (diamonds symbol, $r^2 = 0.57$, $P = 0.13$) with respect to the ratios between populations under different management type (+ symbol, $r^2 = 0.27$, $P = 0.02$).

The bootstrap analysis of the phenogram provides strong support ($P < 0.001$) for differences among populations according to their spatial distribution (Fig. 3). Two groups of populations were clustered together.

Populations C3, M3 and C2, which occupy the western

TABLE 6. Proportion of outcrossing progeny (t_m), maternal fixation index (f), biparental inbreeding (bf), and proportion of individual with exclusively outcrossed progeny (T), for six populations of *Polaskia chichi*pe which different types of management in Tehuacan Valley, Mexico.

Population	N	t_m	f	bf	T
Wild					
W1	9/148	0.898 (0.063)	0.000 (0.000)	0.064 (0.039)	0.22
W2	11/187	0.949 (0.061)	0.005 (0.048)	0.060 (0.046)	0.54
Silvicultural					
M1	13/151	0.890 (0.074)	0.008 (0.062)	0.090 (0.059)	0.42
M3	7/110	0.823 (0.081)	0.000 (0.041)	0.005 (0.061)	0.143
Cultivated					
C1	14/251	0.890 (0.071)	0.007 (0.071)	0.102 (0.060)	0.29
C2	10/120	0.979 (0.045)	0.060 (0.074)	0.066 (0.048)	0.22
Average for management type					
Wild	20/335	0.924 (0.062)	0.003 (0.024)	0.062 (0.042)	0.40
Silvicultural	20/261	0.857 (0.077)	0.004 (0.052)	0.047 (0.060)	0.32
Cultivated	24/371	0.935(0.058)	0.034 (0.073)	0.084 (0.054)	0.26

side of the study area, comprise a first group, and the rest of the populations occupying the eastern side of the area were clustered in a second group. In the second group, the most similar populations were C1 and W2. Populations M1, M2 and W1 occurred at the center of the sampling area and formed a subgroup. Population W3,

also in the second group, is the population with the highest genetic diversity ($H_E = 0.73$). When each population was analyzed for genetic bottleneck under the TPM model with 95% SMM, none of them was significant after 5000 iterations ($P < 0.05$).

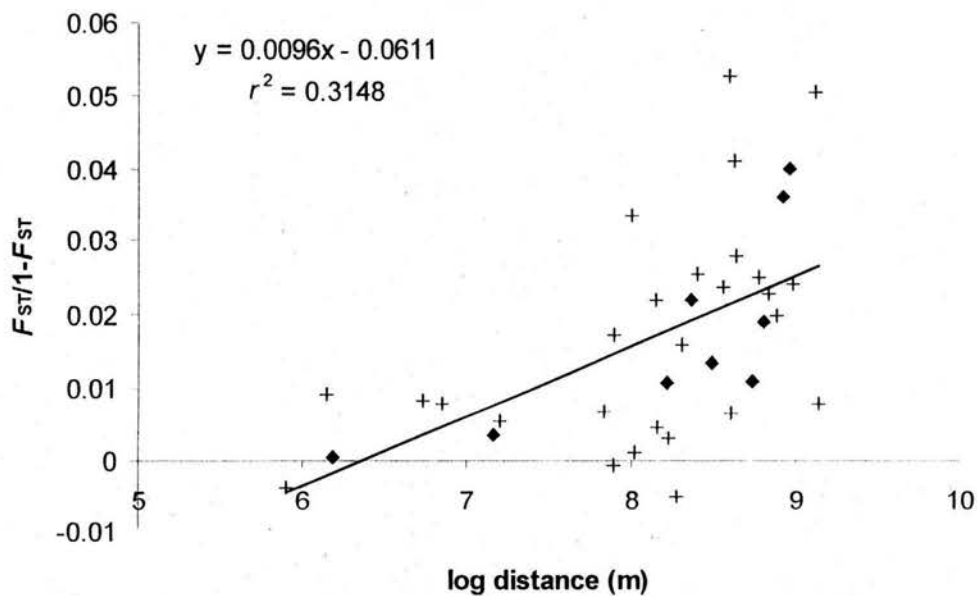


FIG. 2. Relationship between pairwise comparisons of genetic and geographic distance. Multilocus estimates of $F_{ST}/1 - F_{ST}$ are plotted against the euclidian distances (+ symbols for comparisons between populations under different management, diamond symbols for comparisons between populations under the same management type).

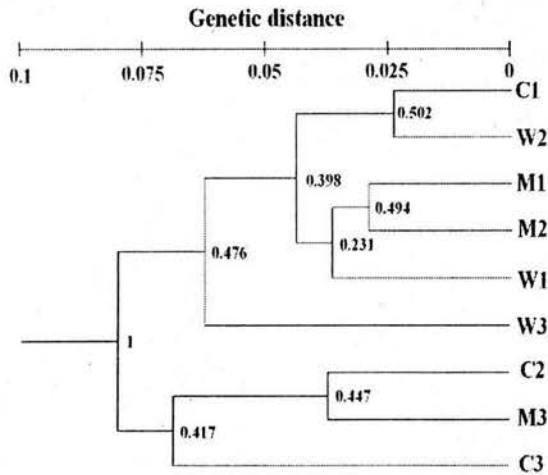


FIG. 3. UPGMA cluster based on Nei's (1972) genetic distances estimated among 9 populations of *Polaskia chichipe* under different levels of management in Tehuacan Valley. Proportions of similar replicates after 10,000 permutations are shown for each clade.

DISCUSSION

Genetic variation found in *P. chichipe* with 5 microsatellite loci is within the range of microsatellite variation reported for other species of long-lived plants with mixed-mating system (0.64-0.85 in some trees according to Byrne et al. 1996; Chase et al. 1996; Aldrich et al. 1998).

Although frequency of vegetative propagation was low (2%), presence of clones in different populations suggests routes of recent dispersal by humans within those populations (in M3 and C2) and between populations (C2-C1, W1 to M3, W3 to M2).

An excess of homozygosity within populations can be due to at least two causes: inbreeding and population subdivision. Positive F_{IS} values found in all populations indicate potential levels of inbreeding that are inconsistent with the mostly outcrossed mating system. However, mating with close relatives could be common, biparental inbreeding contributed significantly to selfing rates. An apparent deficiency of heterozygotes could result from Wahlund effects if there is substantial substructuring of individuals within populations (Hartl and Clark 1997). Because our objective was to compare the genetic diversity among populations and management type, our data are not sufficiently to resolve substructure within populations. But in the study of local substructure and gene flow in *P. chichipe* we found some evidence that support substructure as the cause of heterozygote deficiency (Otero-Arnaiz 2004)

According to Otero-Arnaiz et al. (2003), *P. chichipe* is pollinated by several species of insects with foraging activity frequently restricted to short flights among

flowers on one or few individuals. *P. chichipe* is also likely pollinated by hummingbirds which may fly longer distances than bees but that commonly fly relatively short distances compared with bats, the primary pollinator of other species of columnar cacti. The mixed mating system of *P. chichipe* appears to favor inbreeding associated to the pattern of movement of pollinators among nearest neighbors, but both the selfing and inbreeding coefficient do not vary among populations. However, the proportion of individuals producing selfing progeny varied among management forms, being smaller in wild populations. This means that few individuals in wild populations produce more selfed progeny compared with manipulated populations. This result is consistent with the ones obtained in our previous study on reproductive biology of *P. chichipe*, in which we found that manual self-pollination in cultivated populations was successful in more individuals than in wild populations (Otero-Arnaiz et al. 2003).

Reproduction by seeds seems to be the main way of propagation under natural conditions. This, in turn, favors gene flow among neighboring populations because fleshy fruits of *P. chichipe* are a source of food and water for a variety of birds and bats that act as seed dispersers. Migration between neighboring populations in close proximity, as suggested by the isolation by distance analysis, has contributed to homogenization of genetic variation among populations. Even if migration occurs preferentially within populations under the same management type, neighboring populations under different management types are less differentiated than more distant populations under the same management type because the isolation by distance is avoiding a larger differentiation. This observation is supported by the result that distance explained a higher proportion of differentiation among populations under the same management type than did differentiation among populations under different forms of management.

In theory, the process of domestication is expected to result in significant reductions of genetic variation, as has been observed in most species with signs of advanced levels of domestication (Doebley 1992). Results of our study of *P. chichipe* are generally consistent with this pattern. The highest genetic variation was found in wild populations, whereas in the human manipulated populations, genetic variation was reduced in silviculturally managed populations and intermediate in cultivated populations, although there is non significant differences in the levels of genetic variation. The low genetic differentiation suggests that *P. chichipe* is still in the incipient stages of domestication, despite morphological differences observed among populations under different management type (Carmona and Casas in press), and artificial selection favoring a higher proportion of

individuals with self-pollination (Otero-Arnaiz et al. 2003). Such slight differentiation could be due to the sympatry of the populations allowing for migration among neighboring populations that can balance the effects of artificial selection. Additionally, characteristics of the species, such as long-lived perennial habit, with little vegetative propagation reduce the probability for fixing desirable characters in a population through artificial selection. Within populations, artificial selection can be delayed because people tolerate or to eliminate individual cacti based on their ability to distinguish the desirable characteristics in the plants, which does not occur until after fruit production (6 to 8 years after seed germination, according to Casas and Barbera 2002), only then they decide to tolerate or to eliminate individual plants.

Considering the form of management, a bottleneck associated with the domestication process in *P. chichipe*, would be expected to be stronger under silvicultural management in situ, intermediate in cultivated population and weak or nonexistent in wild populations. We did not detect bottleneck reductions in effective size of populations under any management type. Our results suggest that populations manipulated by humans have not suffered genetic bottlenecks as a result of the constant migration among populations. Alternatively, gene flow could be directional from wild to manipulated populations, avoiding effects of size reductions of manipulated populations. To test these hypotheses, it is necessary to estimate patterns and directionality of gene flow among populations under different management type through direct estimation of pollen and seed flow.

Although *Polaskia chichipe* has a long history of use and management that resulted in morphological differentiation, changes in breeding system and seed germination patterns associated to human management, this is reflected only slightly in genetic differences among populations. Migration and history of populations seems to have intervened to counteract effects of artificial selection over this species.

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GENE FLOW AMONG WILD AND MANAGED POPULATIONS OF *POLASKIA CHICHIFE* AN ENDEMIC COLUMNAR CACTUS IN CENTRAL MEXICO.

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Abstract. Microsatellite markers were used to obtain direct and indirect estimates of gene flow in populations of *Polaskia chichipe* under different management regimes, in order to understand the genetic consequences of gene flow in the evolutionary process of domestication. *P. chichipe* is a columnar cactus endemic to the Tehuacan Valley, Central Mexico. This is under domestication because of its edible fruit. Artificial selection is practiced in silviculturally managed and cultivated populations which are sympatric with wild populations. Morphological, phenological, physiological and reproductive differences between wild and managed populations have been documented in previous studies, apparently associated to human selection. However, strong gene flow may counteract the effects of selection. In this study we used paternity analysis procedures to demonstrate that although most of the pollinations occur among individuals within the same population at distances < 40m, pollen immigration rates (0.27 ± 0.05) are considerable. Heterogeneity in pollen clouds sampled by mother plants ($F_{ST} = 0.12$) indicated non random mating, and is probably due to temporal heterogeneity in pollen movement. *P. chichipe* has a local and regional scale spatial structure that is consistent with isolation by distance model. The similarity of indirect, direct and demographic estimations of neighborhood size (74-250 individuals) suggests that this genetic structure is representative of an equilibrium state. These results suggest that traditional management practices have conserved the genetic resources of this species in situ but also, that gene flow is counteracting the effect of domestication to some degree.

Keywords: columnar cacti, crop evolution, domestication, spatial genetic structure, genetic resources, gene flow, microsatellites, *Polaskia chichipe*, landscape genetics.

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Domestication is an evolutionary process directed by selection conducted by humans to adapt plants and animals to their needs. This process has been conducted for approximately 10,000 yr, and southern Mexico has been identified as one of the main centers of plant domestication (Harlan 1971; Hawkes 1983). In the course of domestication, humans determine morphological, physiological and behavioural changes in the organisms through artificial selection. In plants, morphological changes such as size, colour, flavour of edible parts; physiological changes in fruit maturation, loss of dormancy and faster and uniform germination; and phenological changes as a result of domestication have been documented in a number of plants (Hawkes 1983; Harlan 1992; Evans 1993; Frary and Doganlar 2003).

How fast the domestication process advances, depends on how strong artificial selection operates, as well as on how easy the fixation of desirable characteristics in manipulated populations is. This last aspect may be influenced by life cycle, inter-generation time, and reproductive isolation with respect to populations of wild relatives. Fixation of desirable characters and reproductive isolation is relatively easy through vegetative propagation, self pollination and spatial and/or temporal isolation. However, when wild and domesticated variants are sympatric, are outcrossing, sexually compatible, and their flowering times overlap, gene flow may occur among them (Le Thierry d'Ennequin et al. 2000; Montes-Hernández and Eguiarte 2002; Papa and Gepts 2003). Gene flow may be sufficient to avoid genetic isolation between wild and domesticated populations and only continual artificial

selection would maintain the identity of wild and domesticated populations.

Polaskia chichipe is a columnar cactus endemic to the biosphere reserve Tehuacán-Cuicatlán, Central Mexico, where the use of cacti has been reported to be as old as the first human occupation of the region (Smith 1965, 1967). The mating system of *P. chichipe* is highly outcrossing (90%, Otero-Arnaiz et al. submitted). The species has a generalist system of pollination, conducted by bees, carpenter bees, bumblebees, melliponinae bees, hawkmoths and hummingbirds (Otero-Arnaiz et al. 2003). Although seed dispersal has not been studied, it has been observed that birds and bats consume its fleshy fruits and they and humans are, most likely, the main seed dispersors. *P. chichipe* grows wild as the main component of the vegetation structure in the thorn-scrub forest described as "chichipera" (Valiente-Banuet et al. 2000), over volcanic soils at elevations of 1600-2300 m.

Significant differences between individuals of wild, silvicultural and cultivated populations have been reported for *P. chichipe* by Carmona and Casas (in press) and Otero-Arnaiz et al. (2003) in morphology, mainly in fruit and flower characters (larger fruits with sweeter pulp and more and larger seeds, larger ovaries in cultivated populations), germination patterns (higher percentage and faster germination in cultivated populations), phenology (long blooming and fruit production season in cultivated populations) and reproductive biology (12% of self-compatible breeding system in the wild and 22-27% in managed populations). These differences indicate that domestication process has apparently had a significant influence on the phenotypic structure of populations and that artificial selection is acting on silvicultural populations as well as in cultivated populations favoring vigorous individuals, with larger fruits and more seeds, with self-pollination. Partial isolation due to differences in the peak flowering period of cultivated, silvicultural and wild populations may contribute to in maintaining the phenotypic divergence referred to above (Otero-Arnaiz et al. 2003).

However, genetic differentiation among populations under different management regimes, measured as F_{ST} with microsatellite markers, was significant but slight. Apparently, genetic diversity responds more to geographic distribution and historical gene flow among neighboring populations, and this counteracts the effect of selection through management (Otero-Arnaiz et al. submitted).

In cultivated populations, genetic diversity is as high as in wild populations, probably because people bring new phenotypes from other towns, and this practice may have enriched genetic diversity within home gardens, whereas in silvicultural populations genetic diversity is slightly reduced, but these populations have functioned as bridges for gene flow with neighboring wild and

cultivated populations. Considering all this information, we hypothesized that the slight reduction in genetic diversity observed in manipulated populations of *P. chichipe* is caused by an accumulation of genetic diversity in manipulated populations due to pollen gene flow from wild to manipulated populations. Gene flow may counteract artificial selection for domestication even under a long history of selection. But, in contrast, gene flow contributes to the maintenance of genetic diversity within populations of the species. In this study we analyze the actual distribution and directions of gene flow and the heterogeneity in pollen pools within and among populations and the genetic structure at different spatial levels for cultivated, silvicultural and wild populations of *P. chichipe* in Tehuacan-Cuicatlán Valley. We test the hypothesis of asymmetrical gene flow from wild to manipulated populations and discuss the effect of gene flow in the domestication process and conservation of this species.

The main purposes of this work were: i) to estimate rates of selfing and patterns of pollen movement within and among populations of *P. chichipe*, comparing these estimates among populations under different management types, ii) to estimate variation in pollen dispersal and male reproductive success, iii) to estimate heterogeneity in pollen clouds sampled from mother trees and to explore the relationship with spatial distance among mother trees, and iv) to analyze the genetic structure of populations of *P. chichipe* at different spatial levels, to obtain indirect estimates of localized gene flow and to compare them with direct estimates.

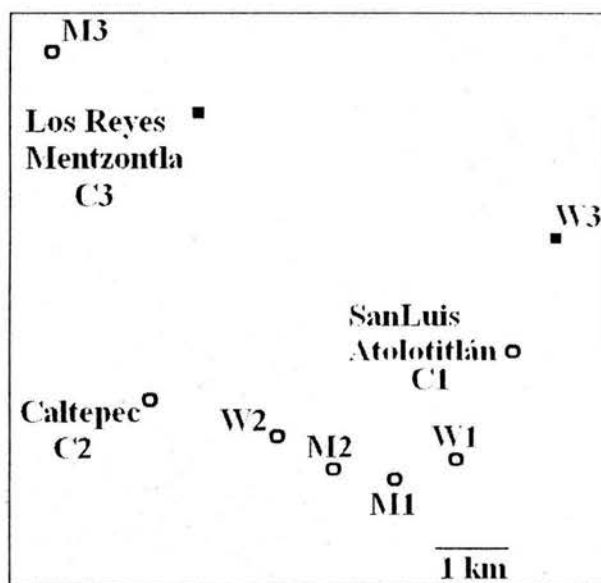


FIG. 1. Distribution of populations studied. Open circles represent the populations in which mother trees were located.

MATERIALS AND METHODS

Sampling and genotyping plant material

The indirect quantification of gene flow was carried on in nine populations under cultivation, silvicultural management and wild populations, all distributed within an area of approximately 80 km² (Fig. 1). We considered these populations as parts of a metapopulation, because the pattern of human management with the creation of new cultivated populations and contributing to fragmentation of others, simulates the dynamics of a metapopulation. Also there is slight genetic differentiation among the populations, with populations presumably linked by gene flow between neighboring populations (Otero-Arnaiz et al. submitted). Distances between populations range from 360 to 9300 m. In each population, 30 reproductive individuals were marked and mapped. For all marked individuals, flower buds were collected and stored at -70°C for DNA extraction. For direct estimation of pollen gene flow seeds of between 5 to 14 adults per population in each of 7 populations were germinated and at least 12 seedlings (15 seedlings per individual on average) were selected for DNA extraction.

Genotyping of all adults and seedlings was conducted with five microsatellite loci (Pchi9, Pchi21, Pchi20, Pchi50, Pchi54) identified specifically for *P. chichipe*, which were described previously (Otero-Arnaiz et al. in press; Otero-Arnaiz et al. submitted). DNA extraction and genotyping was performed as described by Otero-Arnaiz et al. (in press).

Direct estimation of gene flow

Methods of paternity exclusion (Dow and Ashley 1996; Krauss 1999) and maximum likelihood of paternity analysis (Meagher 1986; Meagher and Thompson 1987; Marshall et al. 1998) were used for direct estimation of gene flow. For each reference population, paternity assignment was determined by simple exclusion based on their multilocus genotypes. Male haplotype was inferred by the subtraction of the female contribution from the offspring multilocus genotype. The male haplotype was compared with the possible haplotypes of all individuals, including the maternal plant in each population. In cases where more than one possible pollen donor were assigned to any progeny, the maximum likelihood of paternity obtained as a LOD score (Meagher and Thompson 1987) was estimated through the program CERVUS 2.0 (Marshall et al. 1998). Since significance levels for LOD-scores can not be properly derived analytically, a simulation approach of 10,000 tests was performed to evaluate the significance of the paternity analysis. When more than one individual shared the same LOD score, or when the

significance of the paternity analysis was lower than 80%, paternity was assigned to the spatially nearest individual with highest positive LOD score (Dow and Ashley 1996; Gonzalez-Martínez et al. 2002).

Proportions of selfing and outcrossing, as well as the number of alleles received from pollen donors by each maternal individual and population, were calculated and the differences between management types were compared with a *t*-test. The outcrossing rate, proportions of local gene flow, and immigration of pollen were arcsine-square root transformed before all analyses.

Male reproductive success of each individual was estimated as the proportion of gametes contributed by a particular individual in relation to the total progeny sired within the reference population. This parameter was partitioned (MRS = MSRS + MORS) in male selfing reproductive success (MSRS = $[S_i / \sum_{i=1}^n S_i] \times 100\%$) and male outcrossing reproductive success (MORS = $[O_i / \sum_{i=1}^n H_i] \times 100\%$), where *n* is the number of individuals in the population; *S_i* is the number of selfing male gametes contributed by the *i*th individual; *O_i* is the number of male gametes contributed by the *i*th individual to the total progeny that were assigned to a particular father; and *H_i* is the number of progeny that were assigned to the male *i*; according to He and Smouse (2002). Variation in MORS among individuals was related to the spatial distribution.

Heterogeneity in pollen pools

Male haplotype frequencies were estimated by subtraction of the maternal haplotype from each progeny genotype. For loci in which the mother and progeny were heterozygous for the same alleles, Mendelian inheritance of maternal alleles was assumed, hence allowing the estimation of the frequency of these alleles in the parental pollen pool. To test whether maternal trees spatially closer sample more similar pollen clouds than distant trees, the $F_{ST}/1-F_{ST}$ of pollen clouds was compared with the logarithm of geographic distance among pairs of mother trees, through the program SpaGeDi 1.1 (Hardy and Vekemans 2002).

Indirect estimation of gene dispersal distances

The analytical model based on isolation by distance of populations proposed by Rousset (1997), and extended to test whether migration rate depends on the spatial distance among individuals in a continual population (Hardy and Vekemans 1999; Rousset 2000) was used to estimate gene flow indirectly. Indirect gene flow based on this method can be expressed in terms of Wright's neighborhood size and then compared with direct estimations of the same parameter (Fenster et al. 2003).

To characterize the spatial genetic structure within each population and at the metapopulation level,

considering all the individuals irrespective of their population or management, relative kinship between each pair of individuals was estimated. Relative kinship, according to Rousset (2000), is the difference in ratios of probabilities of identity in state, and it was computed as a correlation coefficient between allele states. In the case of two individuals i and j , the relative kinship coefficient between them can be defined as $F_{ij} = (Q_{ij} - Q_m) / (1 - Q_m)$, where Q_{ij} is the probability of identity in state for random genes from i and j , and Q_m for genes coming from random individuals in the reference population; this estimate is not biased by the presence of rare alleles (Hardy and Vekemans 2002). Relation of kinship coefficients (F_{ij}) with space was analyzed in two ways: i) average F_{ij} were computed for the following distance classes as in a spatial autocorrelation analysis: 0-10, 10-20, 20-30, 30-40, 40-60, 60-80, 80-100, 100-500, 500-1000, 1000-2000, 2000-3000, 3000-4000, 4000-5000, 5000-6000 m. Only the first eight distance classes were represented when the analysis was performed within each population; ii) the estimator F_{ij} computed for each pair of individuals was regressed against the logarithm of the geographic distances between those individuals. When the population is represented by a bidimensional space under isolation by distance in a restricted geographic scale ($\approx \sigma$ to 20σ , where σ is the axial standard deviation of gene dispersal distances), F_{ij} is approximately linearly related to the logarithm of distance (Rousset 1997; Hardy and Vekemans 1999). Then, we regressed F_{ij} values in a restricted and global distance range (as in Fenster et al. 2003), using σ estimated by the direct approach of pollen gene flow to define the restricted distance range. Significance of the average F_{ij} per distance class and

regression slopes was tested by randomization tests, through 10,000 permutations of individuals among locations. All analyses were performed using SpaGeDi 1.1 (Hardy and Vekemans 2002). This method provides an indirect estimation of the neighborhood size, in terms of number of individuals expressing the strength of local genetic drift (Wright 1943). $N_b = 4\pi D\sigma^2$ (where D is the effective population size and σ^2 the variance in gene distance dispersion) was obtained from the regression of the pairwise F_{ij} values between individuals on geographic distance as $N_b = -(-1 - F_{ij}) / \text{slope}$ at different spatial scales (see theoretical analysis in Rousset, 1997, 2000; Hardy and Vekemans, 1999).

RESULTS

Paternity assignment

The five microsatellite markers were highly polymorphic in *P. chichipe*, resulting in theoretical exclusion probabilities of 0.96. Among the 673 seedlings that had their male parent within the population, 564 (0.84) showed the highest LOD score, allowing the designation of a single male parent to all progeny that were the result of local gene movement or self-fertilization. Among these seedlings, 64% of the paternity assignment was successful with a confidence level higher than 80%.

Of the 954 progeny analyzed, 0.73 appear to be derived from matings between individuals within the reference population, while at least 0.27 should be derived from fathers from outside the reference population (Table 1). This is a minimal estimate of the immigration of pollen, since gametes assigned to

TABLE 1. Number of maternal trees, progeny sampled, minimum estimation of long distance gene flow and selfing rate for populations of *P. chichipe* under different management type. Standard error in parenthesis.

	Maternal trees	Offspring	Pollen immigration (se)	Outcrossing mates within population (se)	Selfing
Cultivated					
C1	14	247	0.28 (0.06) ^{nc}	0.66 (0.06) ^a	0.06 (0.03) ^a
C2	9	119	0.33 (0.08) ^{nc}	0.62 (0.07) ^a	0.05 (0.04) ^{ac}
Silvicultural					
M1	7	104	0.14 (0.09) ^{bc}	0.71 (0.08) ^{ab}	0.17 (0.04) ^b
M2	6	44	0.11 (0.09) ^b	0.87 (0.09) ^b	0.03 (0.05) ^a
M3	7	107	0.29 (0.09) ^{nc}	0.56 (0.08) ^a	0.14 (0.04) ^{ab}
Wild					
W1	9	148	0.31 (0.08) ^{ab}	0.53 (0.07) ^a	0.16 (0.04) ^{bc}
W2	11	184	0.35 (0.07) ^a	0.59 (0.06) ^a	0.06 (0.03) ^{ab}
Management					
Cultivated	23	366	0.30 (0.05) ^{ab}	0.65 (0.05) ^{ab}	0.05 (0.02) ^a
Silvicultural	20	255	0.18 (0.05) ^a	0.71 (0.05) ^a	0.12 (0.03) ^a
Wild	20	332	0.33 (0.05) ^b	0.56 (0.05) ^b	0.10 (0.03) ^a
Total	63	953	0.27 (0.05)	0.64 (0.05)	0.09 (0.01)

individuals within the population may have actually originated from individuals located outside of this population. Pollen immigration is relatively high with variation among populations, from 0.11 in M2 to 0.35 in W3. In general, pollen immigration in silviculturally managed populations was significantly lower (0.18) than in wild populations (0.33). Self-fertilization varies from 0.03 to 0.16, with an average of 0.09, without significant differences among management types.

TABLE 2. Mean and variance of outcrossing pollen dispersal within populations; n = number of progeny analysed, Dist. = mean of distance and σ_p = variance in pollen dispersal are showed. M3 population was not considered for this analysis.

Population	n	Dist. (m)	σ_p
C1	165	52	3.93
C2	72	45	2.41
M1	70	46	2.94
M2	35	31	2.16
W1	76	34	2.19
W2	101	46	2.63
Average	519	42	2.71

Pollen dispersal

Mean distance of pollen movement within all the populations sampled was 42 m ($\sigma = 2.7$), showing high variation among individuals and among populations (Table 2). The frequency distribution of pollen dispersal distances for each maternal plant and the mean of all progeny show also a high variation among individuals, especially at short distances. A negative exponential model explains between 54 and 90 % of the average mating events for dispersal distances up to 260 m (Fig. 2). The mating success was higher in all populations at short distances (0 - 40 m).

The male reproductive success (MRS) within populations for each plant ranges from zero (in 24% of the trees) to 23 %. The distribution of frequencies of MRS reveals the differences among individuals, 51% of the individuals having a MRS lower than 5 %, whereas only 7 % of the trees have MRS higher than 10 % (Fig. 3). Differentiation of MORS was found in all populations. Some individuals did not contribute pollen to progenies within their populations, among them some of the maternal plants. Individuals with higher pollen contributions of are located in the center of the stand or are the closest to maternal trees. However, other individuals having similar locations did not have high MORS.

Pollen heterogeneity

Each mother tree receives genetically different pollen clouds. Differentiation among allele frequencies in the pollen clouds of the mother trees is $F_{ST} = 0.12$ and levels

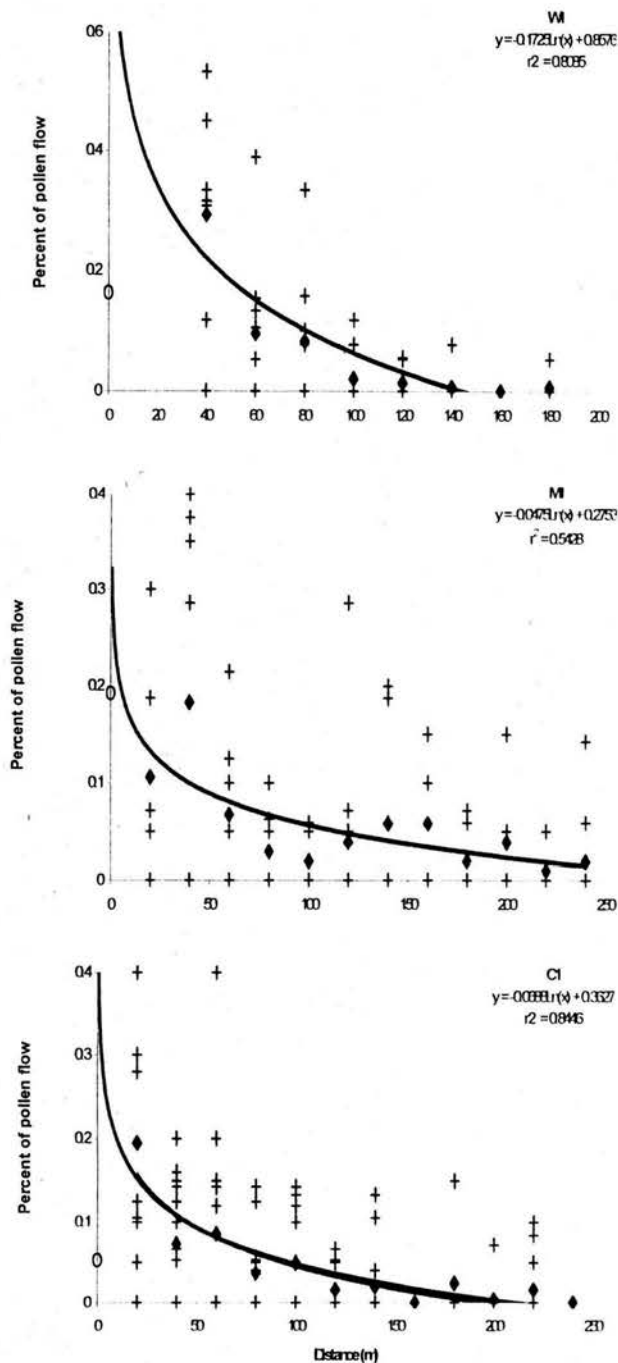


FIG. 2. Distribution of mating frequencies as a function of distance between parental trees for one wild (W1), one silviculturally managed (M1) and one cultivated (C1) populations of *Polaskia chichipe*. Crosses represent mating frequencies for each maternal tree. Diamonds represent the average values over all maternal trees. The line is the fittest exponential curve to the mating frequencies averaged over maternal trees.

TABLE 3. Multilocus differentiation (F_{ST}) in allele frequencies of pollen clouds among different mother trees and among progeny sired by individuals in the reference population and by immigrant pollen. *significant value ($P = 0.05$)

Population	Mother trees	Local vs. immigrant pollen
C1	0.102*	0.035*
C2	0.162*	0.075
M1	0.133*	0.037
M2	0.373*	0.138*
M3	0.112*	0.054*
W1	0.127*	0.061
W2	0.103*	0.045*
All mother trees	0.126*	

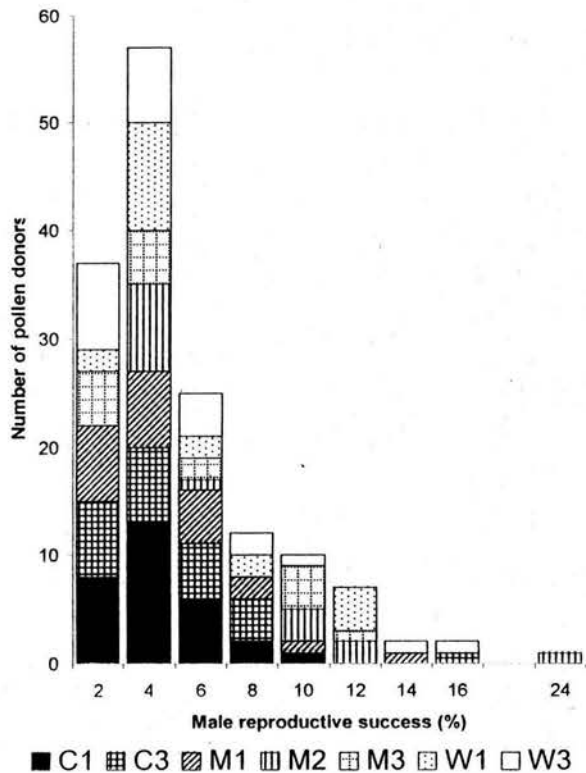


FIG. 3. Frequency distribution of the male reproductive success (MRS) by pollen donors in the reference population

of differentiation varied among populations from 0.10 to 0.16 without a clear pattern among management types (Table 3). Fisher exact test of genetic differentiation showed significant differentiation among all populations ($P < 0.001$), which means that there is no random mating and that different mothers are sampling different pollen pools. When differentiation in pollen clouds in relation to the spatial distance among pairs of mother

trees was tested, no significant relation was found (P of the slope = 0.54). The regression analysis gave a low positive slope of 0.029, which translated to an estimation of neighborhood size of 85 individuals with 95% confidence interval (2se obtained by jackknife over loci) of 26 to infinite. Heterogeneity in pollen sampling by different mothers is probably due to temporal heterogeneity in pollen movement. The analysis of differentiation between pollen allele frequencies within the population and pollen allele frequencies from outside the population shows significant positive values of F_{ST} in more than 50 % of the populations. However, the low estimates indicate a very slight differentiation (Table 3).

Indirect estimations of gene flow

The kinship coefficient between individuals shows an approximately linear relationship with the logarithm of the spatial distance (Figs. 4 and 5). At the metapopulation level (Fig. 4), both regression slopes, global and restricted, were significantly negative with slopes of -0.004 ($P < 0.001$, $r^2 = 0.0015$) and -0.011 ($P < 0.001$, $r^2 = 0.003$), for global and restricted regression, respectively. The variance explained by the regression line is weak, as expected from the high sampling variance for F_{ij} . Average F_{ij} was significantly positive for distance classes < 30 m and for 40 to 60 m ($***P < 0.001$, $**P < 0.01$, $*P < 0.05$ after 10,000 permutation tests, Fig. 4) and significantly negative for distance classes > 4000 m. In the spatial analysis within populations both regressions were also significantly negative: -0.01 ($P < 0.001$, $r^2 = 0.002$) and -0.012 ($P < 0.001$, $r^2 = 0.003$), for global and restricted regression, respectively. Average F_{ij} were significantly positive for the distance classes 20-30 m, and significantly negative for distance > 100 m, but none significantly different to zero in other distance classes (Fig. 5).

Estimations of neighborhood size based on isolation by distance models, were performed using direct estimations of variation in pollen dispersal in the reference population ($\sigma = 2.6$) and among individuals of all populations ($\sigma = 6.6$) according to the spatial scale and from global and restricted regressions of kinship coefficients against logarithm of the spatial distance (Table 4). These indirect estimates are all very similar, ranging from 74 to 250 individuals with confidence intervals widely overlapping, and are also comparable with the estimation of $Nb = 104$ ($38 - \infty$) individuals based on pairwise $F_{ST} / 1 - F_{ST}$ ratios reported previously for these populations (Otero-Arnaiz et al. submitted) and with the estimation of $Nb = 85$ ($26 - \infty$) individuals based in the heterogeneity in pollen clouds. Estimations based on global regression theoretically suffer more bias (Rousset, 1997), as we observed in the confidence intervals in these regressions. At the metapopulation level the distance rate is very large, but the bias can be a

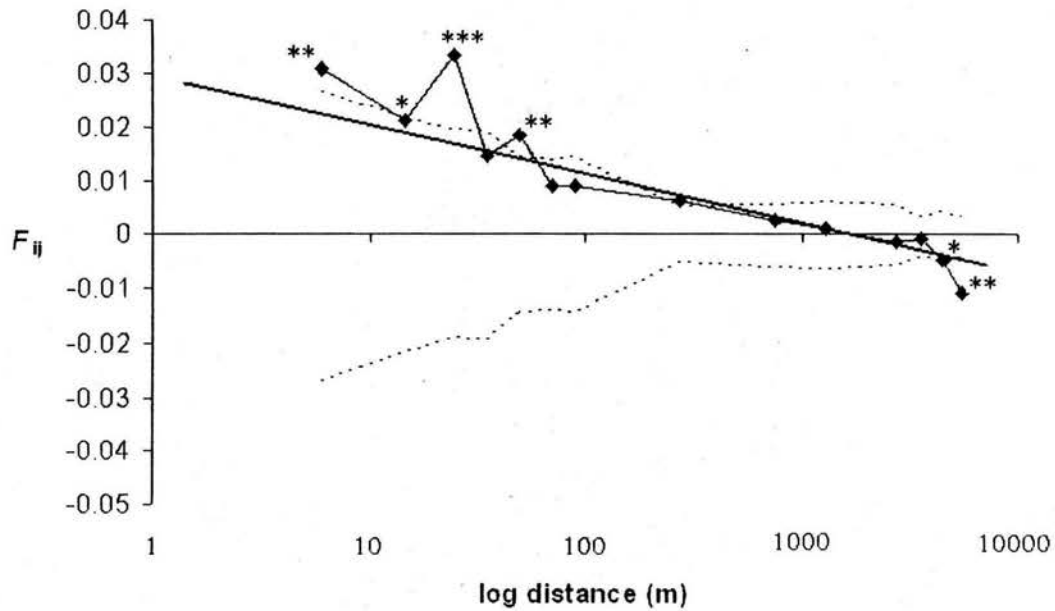


FIG. 4. Spatial genetic structure of *Polaskia chichipe* individuals within and across nine populations. Average values of kinship coefficient for distance intervals (diamonds), 95 % confidence intervals (dotted lines) and significant values after 10,000 permutation tests (** $P < 0.01$, *** $P < 0.001$, * $P < 0.05$) are shown. Global regression (black line) have significant negative slope of -0.004 ($P < 0.001$).

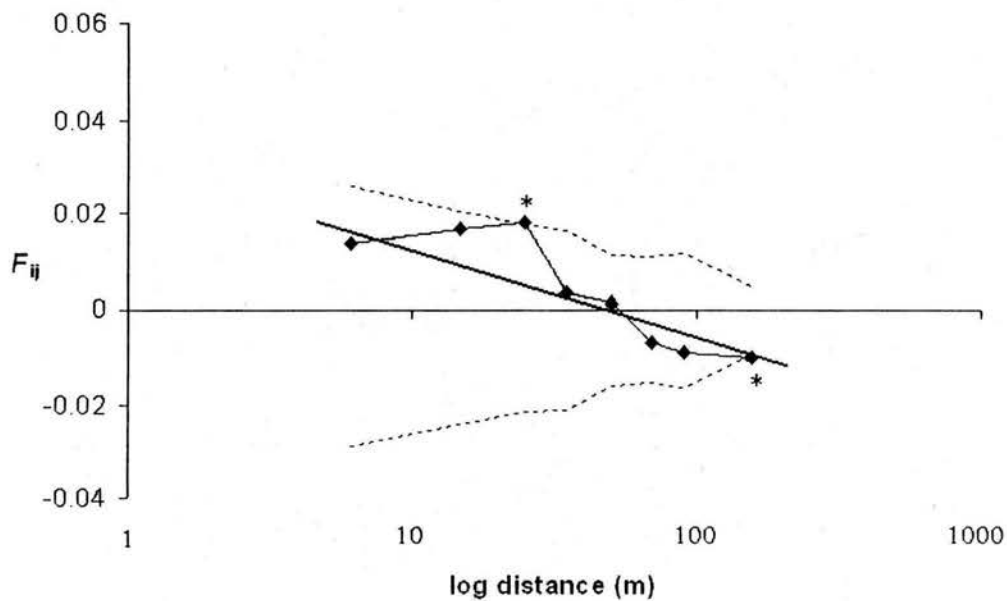


FIG. 5. Relation of kinship coefficients between individuals within populations in the Tehuacan Valley, Mexico. Mean values of kinship coefficient for distance intervals (diamonds), 95 % confidence intervals (dotted lines) and significant values after 10,000 permutation tests (* $P < 0.05$) are shown. Global regression (black line) have significant negative slope of -0.011 ($P < 0.01$).

Table 4. Indirect estimations of gene dispersal (neighborhood size: number of individuals expressing the strength of local genetic drift) across different scales using global and restricted regression for populations and metapopulation of *Polaskia chichipe*. N_b estimates based on average F_{ij} values (see methods). Confidence intervals computed as $\pm 2se$ obtained by jackknife over loci are presented in parenthesis.

	Global	Restricted
Within populations	92 (58-219)	74 (52-132)
Within metapopulation	250 (129-4248)	85 (56-176)

cause of concern. At this level, restricted regression gives a better estimation (Fenster et al., 2003).

DISCUSSION

Paternity analysis and genetic structure

Levels of selfing obtained from paternity analysis did not vary among populations under different management regimes, a result that is in accordance with pollination experiments and mating system analyses reported previously for the species (Otero-Arnaiz et al. 2003; submitted). This result indicates that the lower inbreeding coefficient found in wild populations ($F_{IS} = 0.071$, Otero et al. submitted) compared to silvicultural populations ($F_{IS} = 0.173$, Otero et al. submitted) is caused by outcrossing mating between related individuals in the silvicultural populations. This is also supported by the fact that pollen immigration was significantly lower in silviculturally managed populations than in wild populations (Table 1).

Inference of effective pollen dispersal in *P. chichipe* from paternity analysis indicates a combination of two factors: i) local gene flow where most of the pollen movement occurs between individuals at less than 40 m, observed in the curves of dispersion, and ii) long distance flow for pollen that probably is dispersed several hundreds of meters, supported by the high proportion of pollen immigration to the reference population (27%). These results are consistent with the genetic structure reported previously for the same populations (Otero-Arnaiz et al. submitted). Significant positive values of inbreeding coefficient in all populations most probably represent the impact of local pollen dispersal whereas the long distance of pollen dispersal probably have a profoundly homogenizing influence at the regional level, producing small values of F_{ST} .

Heterogeneity in male success and pollen clouds

Paternity analysis allowed the reconstruction of actual pollination events. Male reproductive success is

irregularly distributed in amount and in space, some individuals contribute more gametes to the progeny than others, indeed some individuals do not contribute to the progeny arrays at all. Apparently, individuals located close to each other have a higher probability to attract pollinators to disperse their pollen compared to isolated individuals. However, this is not the rule, since there are individuals in the center of populations or near maternal trees that did not sire progeny. In addition, some mother trees did not contribute pollen; leading to the speculation that although *P. chichipe* is hermaphrodite, variation in the functional sex could occur. This possibility should be explored further.

Distances among mother trees explain only 3 % of the heterogeneity in pollen clouds. This differentiation may result mainly from the temporal variation in the exposure of pollen, as this is supported by studies of floral phenology in this and other species of *Polaskia* which have shown a great variation among individuals and among populations, as flowering time allows mating among some pairs of trees and excludes other pairs (Cruz and Casas 2002; Otero-Arnaiz et al. 2003). Other factors that can contribute to variation in pollen clouds is mating compatibility, the genetic causes of mating preferences are not known in cactus, but an apparent effect of preferential mating have been detected in controlled crosses in *P. chichipe* (Otero-Arnaiz et al. 2003) and *Stenocereus stellatus* (Casas et al. 1999).

Indirect estimation of gene flow

Indirect estimations of neighborhood size (Table 4) are consistent with the N_b values obtained from the regression analysis between heterogeneity in pollen clouds and distance (pollinator neighborhood of 85 individuals) and from demographic studies in progress (88 individuals, according to Berenice Farfan, personal communication). This information suggests that the observed local genetic structure is representative of an equilibrium state and that a significant pattern of isolation by distance is responsible for the spatial genetic variation at the microsatellite loci in *P. chichipe*. Such consistence among different estimations of N_b may depend on the geographic scale of the study. In fact, detection of isolation by distance is a matter of scale, depending on the range of distances analysed, and on the variance of the estimators which is probably lower at short distances (Rousset 1997). In a local scale stochastic equilibrium is approached more rapidly (Slatkin 1993), and spatial variation in demographic parameters are not represented at short distances.

The spatial genetic structure observed in *P. chichipe*, corresponds to a model of isolation by distance, and it was not limited to the genetic relationships among individuals within populations or within the metapopulation. These authors also reported a significant relationship between differentiation among

pairs of populations and the logarithm of distance (Otero-Arnaiz et al. in prep.). These results are also consistent with observations on pollination biology (Otero-Arnaiz et al. 2003). *P. chichipe* is pollinated by several bees species with a foraging activity more frequently restricted to short flights to flowers of one or a few neighboring individuals and possibly also by hummingbirds which may fly longer distances than bees (>5km, according to Arizmendi 2001) but that more frequently fly short distances.

In sum, moderate levels of gene flow in *P. chichipe* avoid genetic isolation between wild and domesticated populations. The continual artificial selection practiced by farmers maintains the identity of wild and domesticated populations and contributes to the conservation *in situ* of this endemic species. *P. chichipe*, is a perennial, long lived, highly outcrossing, with generalist pollination system, with sympatric populations under different levels of domestication. Species that share similar characteristics of the natural history and management histories may also have similar patterns of gene flow and differentiation among cultivated and wild populations.

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DISCUSIÓN GENERAL

LOS PROCESOS DE DOMESTICACIÓN EN

POLASKIA CHICHIPE.

Adriana Otero Arnaiz

Las poblaciones de *Polaskia chichipe* del Valle de Tehuacán bajo manejo silvícola, cultivadas o silvestres son simpátricas. Existen diferencias morfológicas entre poblaciones bajo diferente manejo en caracteres que, basados en información etnobotánica, fueron identificados como blancos de selección artificial, principalmente las características del fruto (Carmona y Casas en prensa). Considerando que dentro de una misma población existen variantes fenotípicas y puesto que en las poblaciones silvestres pueden identificarse fenotipos de poblaciones silvícolas o cultivadas y viceversa, Carmona y Casas (en prensa) consideran que la variación morfológica encontrada debe tener un importante componente genético. La hipótesis de los autores mencionados es que la divergencia morfológica documentada se debe preponderantemente a la selección artificial que favorece los fenotipos con mejores atributos utilitarios dentro de las poblaciones manipuladas.

Para entender cómo se mantiene la divergencia morfológica observada entre poblaciones silvestres, manejadas *in situ* y cultivadas, en la presente investigación se estudió el sistema de apareamiento de la especie. Se buscó establecer si existe algún tipo de aislamiento reproductivo entre poblaciones bajo diferente manejo y si la manipulación humana ha modificado los patrones reproductivos en las poblaciones de plantas que manipula. Asimismo, se cuantificó la estructura genética de poblaciones bajo diferente manejo con el fin de evaluar el efecto de la manipulación humana sobre la diversidad genética de las poblaciones. Se evaluó la relación entre la distribución geográfica de las poblaciones e individuos con su variación genética con el fin de establecer de qué manera el sistema de apareamiento está favoreciendo o no la fijación de caracteres seleccionados. Finalmente,

se analizó el efecto del flujo génico actual e histórico con el fin de conocer de qué manera este factor puede balancear los efectos de la selección artificial. La integración de los avances en cada uno de estos aspectos permite analizar las consecuencias evolutivas que ha tenido la domesticación de esta especie, así como también aporta información que sienta las bases para desarrollar estrategias de manejo para mantener la integridad biológica de la especie, como se discute en este capítulo.

Biología reproductiva

La mayoría de las cactáceas columnares son parcial o totalmente dependientes de murciélagos para su polinización (Petit 1995; Fleming et al. 1996; Sahley 1996; Valiente-Banuet et al. 1996a; 1996b; Nassar et al. 1997; Valiente-Banuet et al. 1997a; 1997b; Casas et al. 1999), lo que permite un amplio movimiento de polen entre poblaciones debido a las largas distancias que pueden volar estos vectores, determinando generalmente un elevado flujo génico entre poblaciones (Nason et al. 2002; Nassar et al. 2002, 2003). Sin embargo, en *P. chichipe* se observó que la antésis es principalmente diurna y sus flores son visitadas por insectos de cinco órdenes taxonómicos (himenópteros, dípteros, coleópteros, hemípteros y lepidópteros) así como por tres especies de aves, lo mismo en poblaciones silvestres que en las poblaciones silvícolas y cultivadas (Otero-Arnaiz et al. 2003). Algunos de los visitantes florales registrados pueden volar distancias mayores a las que separan las poblaciones. Los colibríes, por ejemplo, pueden volar distancias > 5.5 km/d (Arizmendi 2001), mientras que el rango de distancias entre las poblaciones es de 0.5 a 9 km, con un promedio de 4.4 km, lo que en primera instancia sugiere que las barreras reproductivas

espaciales son poco probables. No obstante, se observó que frecuentemente los visitantes florales presentan un patrón de forrajeo de viajes cortos, visitando con mayor frecuencia flores del mismo individuo o de individuos cercanos; además, se desconoce aún el tiempo en que permanece viable el polen de *P. chichipe* y el tiempo que duran los viajes largos de los visitantes florales, por lo que la información generada en esta parte del estudio no permite ser concluyente en cuanto a la existencia o no de barreras espaciales al intercambio de polen entre las poblaciones analizadas.

Los resultados del estudio sobre fenología floral las poblaciones de *P. chichipe* con diferente manejo indican que existe un traslape de tres meses en la producción de flores. Sin embargo, hay un desfase de un mes en el pico de floración entre poblaciones con diferente manejo; en las poblaciones silvestres se presenta en febrero, en las poblaciones con manejo silvícola en marzo y en las cultivadas en abril, cuando ya no hay flores ni en las poblaciones con manejo silvícola ni en las silvestres, lo cual parece determinar una barrera temporal, al menos parcial, al movimiento de polen entre estas poblaciones (véase Fig. 4, Otero-Arnaiz et al. 2003, Capítulo 1). Por otra parte, los resultados de experimentos de cruza entre individuos de diferentes poblaciones sugieren la presencia de un mecanismo de aislamiento por compatibilidad de polen, ya que la producción de frutos fue más exitosa cuando el polen provenía de la población de referencia que cuando el polen provenía de poblaciones con diferente manejo (véase Tabla 7, Otero-Arnaiz et al. 2003, Capítulo 1). Adicionalmente, la existencia de mecanismos de autopolinización que se registró en *P. chichipe* y que se discutirán más adelante también constituye otro factor de aislamiento reproductivo parcial entre poblaciones. Entonces, en relación a la pregunta de si existe aislamiento reproductivo entre poblaciones, el estudio de biología reproductiva permite visualizar que: 1) las

barreras espaciales no son descartables, pero la evidencia disponible no las apoya. Es necesario analizar con detalle los patrones de forrajeo de los visitantes florales y analizar el tiempo que permanece viable el polen; 2) las barreras temporales son posibles, pero solo parcialmente. Es necesario, sin embargo, analizar si el desfase en los picos de floración observados se mantienen año con año o si existe diferenciación temporal entre períodos reproductivos en este comportamiento, como ha sido reportado para *Stenocereus queretaroensis* (Castillo Landero 2003); 3) el aislamiento por compatibilidad de polen opera también parcialmente, pues aunque son más exitosas las cruza entre individuos de una misma población, las cruza con individuos de otras poblaciones son probables. Faltaría, sin embargo, tomar en cuenta la posible influencia del tiempo de viabilidad del polen en estos resultados; 4) el aislamiento por autocompatibilidad es también parcial, ya que sólo una proporción de los individuos de cada población presentó esta característica, además de que el éxito reproductivo fue mayor por entrecruzamiento. Es necesario, sin embargo, analizar qué tan plástica es la autocompatibilidad en diferentes momentos del año y entre períodos reproductivos.

En resumen, el estudio sobre biología reproductiva de *P. chichipe* no es concluyente en cuanto a la existencia de barreras reproductivas espaciales, pero sugiere la presencia de barreras temporales parciales que, junto con la presencia de autocompatibilidad, pueden estar contribuyendo al mantenimiento de la variación morfológica entre poblaciones bajo diferente tipo de manejo y a su vez generando una estructura genética de las poblaciones.

Hasta antes del presente estudio se habían reportado sistemas de apareamiento autoincompatibles para casi todas las especies de cactáceas columnares del Valle de Tehuacán (Valiente-Banuet et al. 1997a; 1997b; Casas et

al. 1999; Cruz y Casas 2002; Oaxaca-Villa et al. en prensa). Las especies del género *Polaskia*, a diferencia del resto de las cactáceas columnares del Valle de Tehuacan, presentan un elevado entrecruzamiento, pero también éxito en cruces de autopolinización (Cruz y Casas 2002; Otero-Arnaiz et al. 2003). En numerosas especies de plantas el proceso de domesticación ha seleccionado individuos autocompatibles, que aseguren la producción de frutos aún en ausencia de polinizadores (Rowlands 1964; Hawkes 1983; Proctor et al. 1996). En el presente estudio, al analizar si la selección artificial ha producido cambios en el sistema de apareamiento entre poblaciones manipuladas por el hombre y poblaciones silvestres en *P. chichipe*, se encontró una importante diferencia en el sistema de apareamiento en relación con el manejo (Otero-Arnaiz et al. 2003). La autopolinización fue exitosa en un mayor número de individuos de poblaciones manipuladas por el hombre (en 43-62%) que en las poblaciones silvestres (17-19%), lo que indica que la posibilidad de mantener o aumentar la producción de frutos en individuos con autopolinización, aún en periodos de baja disponibilidad de polen, seguramente ha propiciado la selección artificial a favor de individuos autocompatibles en las poblaciones bajo silvicultura y cultivadas.

P. chichipe es capaz de producir frutos por autopolinización (21% en autopolinización manual), pero la producción de frutos es mayor cuando la polinización es por entrecruzamiento (43% en polinización cruzada manual), lo cual garantiza el mantenimiento de la heterocigosidad.

En la polinización cruzada, la abeja carpintera *Xilocopa mexicanorum* parece ser uno de los más probables polinizadores del espectro de visitantes florales locales, ya que estuvo presente tanto en invierno como en primavera y se mantuvo siempre con una alta frecuencia de visitas. Por otro lado, *A. mellifera* fue la segunda especie con mayor frecuencia de visitas; esta es

una especie exótica que puede estar desplazando a los polinizadores nativos y que promueve la geitonogamia debido a su forrajeo de limitada movilidad (Herrera 1987; Eynard y Galetto 2002). Para mantener la producción de frutos, así como la diversidad genética de la especie, es necesario promover la conservación de las poblaciones de polinizadores nativos, como *X. mexicanorum*, así como preservar los parches de *P. chichipe* y otras especies columnares que permitan el movimiento de estas abejas carpinteras.

Los estudios de la biología reproductiva efectuados aportan elementos no sólo para entender el proceso de domesticación, sino para otros ámbitos del conocimiento ecológico. Este es el caso de la información registrada sobre los patrones de visitantes florales en invierno y primavera. *P. chichipe* presenta una variación estacional en la duración de la antésis, así como en la diversidad de especies de visitantes florales.

Durante invierno las flores permanecen abiertas por 28 horas y las visitan 7 especies de visitantes florales, mientras que en primavera permanecen abiertas sólo por 13 horas y las visitan 17 especies de visitantes florales. No sabemos si esta variación en el tiempo de apertura de la flor responde a cambios en la disponibilidad de polinizadores o a factores ambientales como la temperatura o la intensidad de la radiación solar. Sin embargo, esta variación está generando a su vez variación temporal en la disponibilidad de polen.

En la mayoría de los estudios de las interacciones de las plantas con sus polinizadores se ha considerado la especialización del sistema de polinización como principal tendencia evolutiva. Sin embargo, recientemente diversos estudios han mostrado que la generalización de los sistemas de polinización es tan frecuente como la especialización, y los estudios experimentales del sistema de polinización indican que la mayoría de las angiospermas son polinizadas por varias especies de insectos y que la mayoría de los

visitantes florales frecuentemente visitan diferentes especies de plantas (Fishbein y Venable 1996; Waser et al. 1996; Johnson y Steiner 2000).

La interacción mutualista entre *P. chichipe* y sus polinizadores puede considerarse como un sistema generalista debido a: i) las características de la flor que permiten a las diferentes especies de insectos y colibríes que las visitan obtener polen o néctar y actuar como polinizadores potenciales, ii) los visitantes florales son taxonómicamente diversos, incluyendo cinco órdenes de insectos y cuatro especies de aves y iii) la variación temporal en visitantes florales hace inconstantes las presiones de selección de los polinizadores más eficientes, por lo que la especialización de características florales para atraer a un polinizador particular es poco probable (Fishbein y Venable 1996; Waser et al. 1996; Johnson y Steiner 2000; Silva-Montellano y Eguiarte 2003).

Estructura genética

La información etnobotánica en relación con las formas de manejo y la información sobre biología reproductiva, particularmente en cuanto a la existencia de barreras reproductivas parciales temporales, así como de compatibilidad diferencial de polen, fueron la base de las hipótesis en cuanto a los niveles de variación y a la estructura genética de *P. chichipe*. El manejo implica una selección a favor de fenotipos deseables y la eliminación de otros por los campesinos en las poblaciones silvícolas. También incluye el establecimiento de poblaciones cultivadas a partir de una fracción de individuos con fenotipos deseables de poblaciones cercanas al poblado, pero adicionalmente la introducción de fenotipos de otros poblados. Por ello se hipotetizó que deberíamos encontrar una reducción de la variación genética en las poblaciones manipuladas por la gente con respecto a las poblaciones silvestres (Doebley

1992). Adicionalmente, se esperaba que tal reducción fuera mayor en las poblaciones silvícolas que en las cultivadas. Considerando también los procesos que originaron las poblaciones manejadas, se supuso que la reducción drástica en el número efectivo de individuos en las poblaciones silvícolas y cultivadas podría reflejarse en su variación genética, al generar cuellos de botella, lo cual ha sido reportado para otras especies bajo domesticación (Ladizinsky 1985). Se esperaba que los cuellos de botella fueran más drásticos en las poblaciones silvícolas.

Por otro lado, si las barreras parciales debidas a factores temporales y de compatibilidad de polen pueden generar un aislamiento en la dispersión de polen, se esperaba que esto se reflejara en una estructura genética poblacional. Finalmente, tomando en cuenta que el estudio sobre biología reproductiva no fue concluyente con respecto a barreras espaciales a la reproducción, se consideró que el estudio de la estructura genética, en relación con la distribución geográfica de las poblaciones, permitiría generar evidencia que probara la hipótesis de aislamiento reproductivo espacial.

A pesar de ser endémica a un área geográfica restringida, *P. chichipe* presenta un nivel elevado de variación genética ($H_T = 0.658 \pm 0.026$). Posibles explicaciones de este elevado nivel de variación genética en la especie podrían encontrarse en el origen y evolución de la especie, la existencia de tamaños de población relativamente grandes y en la naturaleza del sistema de apareamiento. El género *Polaskia* se distribuye en el Valle de Tehuacán-Cuicatlán, y el género filogenéticamente más cercano es *Escontria* (Gibson y Horak 1978; Gibbons et al. 1986; Cornejo y Simpson 1997; Cota y Wallace 1997). El género *Escontria* comparte la distribución de *Polaskia*, aunque además se distribuye en la Costa Pacífica y en la depresión del río Balsas (Dávila-Aranda et al. 2002). Es posible que el ancestro común de este complejo fuera

genéticamente diverso y que la actual variación genética detectada en este estudio sea un reflejo de esa historia. Por otro lado, los niveles elevados de variación genética pueden también ser causados por hibridización interespecifica. Aunque hasta el momento no se han llevado a cabo estudios que confirmen la hibridización entre *P. chichipe* y otras especies, la simpatria de sus poblaciones en el Valle de Tehuacán-Cuicatlán con *P. chende*, *E. chiotilla* y *Myrtilocactus schenkii*, especies con las que mantiene cercanía filogenética (Gibson y Horak 1978; Gibbons et al. 1986; Cornejo y Simpson 1997; Cota y Wallace 1997; Terrazas y Loza-Cornejo 2002), así como el transape del período de floración en todas ellas¹; y el hecho de que comparten polinizadores diurnos, posibilita la hibridización, aunque se requieren estudios que comprueben esta hipótesis.

Para mantener los elevados niveles de variación genética original, se requiere que las poblaciones de *P. chichipe* se mantengan en un tamaño relativamente grande e interconectadas a través de su historia evolutiva. Aún cuando las poblaciones actuales de *P. chichipe* presentan una restricción edáfica asociadas a suelos volcánicos, los datos genéticos indican un elevado flujo génico histórico entre poblaciones ($Nm = 8$ individuos), por lo que es probable que las poblaciones hayan permanecido interconectadas, lo cual es apoyado también por el hecho de no encontrar evidencia de que hayan ocurrido cuellos de botella en ninguna de las poblaciones estudiadas.

P. chichipe presenta un elevado entrecruzamiento y bajos niveles de autocompatibilidad, con una mayor producción de frutos y frutos con mayor número de semillas cuando la polinización es cruzada (Otero-

Arnaiz et al. 2003), lo cual seguramente juega un papel importante en el mantenimiento de la variación genética de la especie.

Aun cuando *P. chichipe* presenta una elevada diversidad genética, se observó un déficit de heterocigosis con respecto a lo esperado bajo equilibrio de Hardy-Weinberg en todas las poblaciones. Este déficit indica niveles potenciales de endogamia que podrían parecer inconsistentes con las características del sistema de apareamiento que se documentaron para la especie en este estudio. Así, aunque el sistema de *P. chichipe* es mixto, presenta un elevado nivel de entrecruzamiento (Otero-Arnaiz et al. 2003). No obstante, el déficit de heterocigotos puede deberse a endogamia biparental, entre individuos emparentados. Una segunda explicación de la deficiencia de heterocigosis podría ser la existencia de una subestructura de los individuos dentro de las poblaciones y, por lo tanto, la ocurrencia del efecto Wahlund (Hartl y Clark 1997). Aunque en esta parte del trabajo no se analizó la estructura intrapoblacional, en el estudio de los patrones de flujo génico entre poblaciones se generó evidencia que apoya que la subestructura es una causa posible de la deficiencia en heterocigotos.

El patrón de variación genética encontrado confirmó la hipótesis planteada inicialmente. Se encontró una mayor reducción de variación genética en las poblaciones silvícolas ($H_E = 0.621 \pm 0.054$) que en las cultivadas ($H_E = 0.660 \pm 0.039$), y la variación genética fue más reducida en estos dos tipos de poblaciones manipuladas con respecto a las poblaciones silvestres ($H_E = 0.683 \pm 0.043$), aunque no hay diferencias significativas entre estas estimaciones. Por otro lado, la diferenciación entre poblaciones, aunque significativa, fue baja ($F_{ST} = 0.015$, $P < 0.001$), y aún más baja y levemente significativa entre tipos de manejo ($F_{ST} = 0.005$, $P < 0.05$), esto es de esperarse debido al origen común de las poblaciones; pero además, la débil

¹ *P. chende* presenta el pico de floración de enero a marzo, *E. chiotilla* florece durante todo el año, presentando un pico de floración en febrero y otro en noviembre, mientras que *P. chichipe* presenta su pico de floración de febrero a abril

diferenciación entre poblaciones por tipo de manejo sugiere la existencia de un substancial flujo génico entre las poblaciones cercanas bajo diferente manejo. Esta conclusión se apoya también en el hecho de que no se observaron efectos de una reducción en el tamaño poblacional o cuellos de botella en las poblaciones manipuladas, como podría esperarse debido al manejo humano. Adicionalmente, el efecto observado de la distancia geográfica sobre la diferenciación genética de las poblaciones, también apoya esta conclusión.

Las poblaciones de *P. chichipe* estudiadas presentaron un aislamiento genético por distancia; esto es, que poblaciones más alejadas espacialmente presentan una diferenciación genética mayor. Al parecer, el flujo génico restringido a las poblaciones vecinas contrarresta los efectos de la deriva génica en la variación genética neutral, evitando una diferenciación mayor entre poblaciones bajo diferente manejo. Por otro lado, las características de historia de vida de la especie, con un periodo prolongado de crecimiento vegetativo antes de su primera reproducción, permiten la acumulación de diversidad genética y evita el efecto de fundador en el caso de las poblaciones manejadas. La baja diferenciación observada entre poblaciones sugiere además que el conjunto de poblaciones estudiadas constituye de hecho una metapoblación, en la que las poblaciones se han mantenido interconectadas por flujo génico (Harding y McNamara 2002). Los bajos niveles de diferenciación (F_{ST}) pueden explicarse, al menos en parte, por la distancia entre estas poblaciones, y en parte también por una selección artificial constante.

Los resultados del estudio de la estructura genética de las poblaciones indican que *P. chichipe* probablemente ha permanecido con tamaños de población relativamente grandes a lo largo de su historia o bien, que las poblaciones han permanecido interconectadas al menos en un pasado reciente. Por ello, una reducción severa del tamaño poblacional podría tener efectos deletéreos.

Tales reducciones podrían alterar el sistema de apareamiento, aumentando los niveles de autopolinización o de apareamientos entre individuos emparentados, lo que incrementaría la tasa de endogamia y potencialmente una depresión por endogamia. De igual forma, si se interrumpe el flujo génico entre las poblaciones podría haber una pérdida de la diversidad genética por efecto de la deriva génica e incrementar la endogamia (Young et al. 1996). Aparentemente, el manejo humano de estas poblaciones ha determinado una reducción de la variación genética; sin embargo, esta reducción no ha sido significativa y la diferenciación entre poblaciones es muy baja, debido a la simpatria de las poblaciones que ha permitido un elevado flujo génico entre poblaciones vecinas bajo diferente manejo. Consecuentemente, para los propósitos de conservar esta especie, es necesario mantener el tamaño actual de las poblaciones, así como las conexiones entre ellas, para de esta manera mantener los niveles elevados de variación genética y evitar efectos deletéreos. Adicionalmente a la protección de las poblaciones existentes, sería recomendable enriquecer, a partir de propágulos de poblaciones silvestres, a las poblaciones bajo manejo silvícola que presentan una reducción en la variación genética y que además sirven de puente al flujo génico entre poblaciones cultivadas y silvestres.

Flujo génico

Para evaluar el efecto del manejo en las poblaciones de *P. chichipe* es importante, además de conocer la variación morfológica, la biología reproductiva y la variación genética de sus poblaciones, entender cómo procesos como el flujo génico están moldeando la variación genética de la especie. Como factor de procesos evolutivos, el flujo génico puede introducir nuevos alelos a las poblaciones y es la mayor fuerza que

actúa contrarrestando la divergencia genética entre poblaciones (Hartl y Clark 1997).

El estudio de la estructura genética espacial permitió la estimación del flujo génico histórico, mientras que las estimaciones del flujo génico actual generaron información en cuanto a: i) la tasa de entrecruzamiento, ii) las distancias de los apareamientos y curvas de dispersión de polen, iii) la variación en el éxito reproductivo masculino, iv) la variación en el polen muestreado por plantas maternas, v) el flujo génico asimétrico entre poblaciones bajo diferente manejo. La comparación entre ambas estimaciones de flujo génico permite hacer inferencias sobre la estabilidad genética de las poblaciones analizadas.

La tasa de entrecruzamiento obtenida con datos genéticos confirma lo observado mediante los experimentos de polinización. *P. chichipe* es una especie con un elevado nivel de entrecruzamiento ($t = 0.91$) que no varía entre poblaciones bajo diferente manejo. Pero el número de individuos con autopolinización es mayor en las poblaciones manipuladas que en las poblaciones silvestres (0.68 y 0.74 en las poblaciones bajo manejo silvícola y cultivadas, respectivamente, y 0.60 en las poblaciones silvestres).

Los niveles de endogamia, estimados como F_{IS} , fueron mayores en las poblaciones manipuladas que en las silvestres y fue en las primeras (especialmente en las poblaciones bajo manejo silvícola) en las que la migración de polen externo fue menor, lo que indica que las diferencias en endogamia responden a un mayor apareamiento entre individuos relacionados más que a una mayor producción de progenie por autopolinización.

En todas las poblaciones se encontraron valores positivos de F_{IS} , lo cual, además de responder a una subestructura poblacional, concuerda con el patrón de dispersión de polen, el cual responde a un modelo logarítmico negativo con una mayor dispersión en distancias de entre 20 y 40 m. Por otro lado, los bajos

niveles de diferenciación entre poblaciones pueden explicarse por el flujo de polen a largas distancias, ya que alrededor del 30% de la progenie fue resultado de la fecundación por polen inmigrante.

Se observó una alta variación en el sistema de apareamiento entre individuos, tanto en la contribución de polen por cada individuo como en el polen muestreado por cada árbol materno. Esta variación puede responder a la heterogeneidad temporal en la exposición de polen, lo que concuerda con la variación en la fenología floral observada entre poblaciones bajo diferente manejo. Además, parece relacionarse con características individuales en cuanto al sexo funcional, ya que se detectaron individuos con un alto éxito reproductivo femenino y nulo éxito masculino, lo que lleva a especular sobre la posibilidad de un ginodioicismo funcional (presencia de individuos hermafroditas y femeninos en una población). Estudios morfológicos y funcionales posteriores serán de gran importancia para probar esta hipótesis. Variaciones del sistema reproductivo hermafrodita han sido reportadas en otras especies de cactáceas columnares como en poblaciones de *Pachycereus pringlei* que presenta ginodioicismo y trioicismo en diferentes áreas de su distribución (Fleming et al. 1998), así como en *Neobuxbaumia mezcalensis* que presenta un sistema de apareamiento androdioico, es decir, poblaciones con individuos masculinos y hermafroditas (Valiente-Banuet et al. 1997b). Consecuentemente, la detección de ginodioicismo funcional y la documentación de los mecanismos que lo determinan podría arrojar importante información para entender la evolución del dioicismo en este grupo de plantas.

El flujo de polen entre poblaciones bajo diferente manejo fue asimétrico, siendo mayor de poblaciones silvestres a cultivadas y de cultivadas a poblaciones bajo manejo silvícola, lo cual se relaciona con la distancia entre poblaciones. Las consecuencias de esta asimetría

en el flujo génico merecen atención, ya que los resultados de los experimentos de cruas mostraron una reducción en la producción de semillas en las poblaciones cultivadas cuando la fuente de polen fue una población silvestre. Si estas semillas germinan y se establecen en condiciones naturales, como lo hicieron bajo condiciones controladas, aún en número reducido, el flujo génico asimétrico estará contribuyendo al mantenimiento de la diversidad genética en las poblaciones manejadas.

La estructura genética espacial de *P. chichipe* responde al modelo de aislamiento por distancia, no sólo en el análisis entre poblaciones, sino también en el análisis entre individuos dentro de las poblaciones y dentro de la metapoblación. Este patrón de variación genética neutral en *P. chichipe* refleja una diferenciación continua. Por ello es difícil determinar unidades de conservación del potencial evolutivo de grupos discretos o poblaciones locales. Alternativamente, como prioridad para la conservación de esta especie endémica debe promoverse el mantenimiento y enriquecimiento de las poblaciones bajo manejo *in situ*, y mantener la continuidad espacial de las poblaciones. El manejo tradicional bajo el que se encuentran algunas especies de cactáceas columnares ha mantenido, como en el caso de *P. chichipe*, o incluso aumentado la diversidad genética, como en poblaciones de *Stenocereus stellatus*, especie en la que se encontró un aumento en la diversidad morfológica y genética en las poblaciones bajo manejo con respecto a las poblaciones silvestres (Casas et al. enviado). El entendimiento del papel que juegan estas formas tradicionales en el mantenimiento de la diversidad genética es particularmente importante en el presente, cuando algunas de estas prácticas tienden a abandonarse y se requiere su revaloración para recuperarlas.

Finalmente, si bien la conservación de la diversidad genética neutral mantiene el potencial evolutivo de la

especie e incrementa su posibilidad de persistencia, es importante tomar en cuenta que el estudio de genética de poblaciones se llevó a cabo con marcadores neutrales, y que aún es necesario evaluar la variación para caracteres adaptativos y fenotípicos. Estos caracteres podrían revelar un patrón distinto de diferenciación genética y deben ser tomados en cuenta también para entender el proceso de domesticación de *P. chichipe*. Merilä y Crnokrak (2001), por ejemplo, encontraron una correlación significativa entre la diversidad en caracteres cuantitativos y marcadores moleculares, pero la diferenciación en caracteres cuantitativos fue mayor que en los marcadores moleculares, indicando un efecto de la selección sobre tales rasgos. Recientemente, algunos autores (McKay y Latta 2002; van Tienderen et al. 2002) han sugerido enriquecer la información sobre diversidad genética obtenida con marcadores neutrales mediante información obtenida con genes que codifiquen para variantes ecológicas relevantes o claramente bajo selección. Esta no es una tarea fácil, ya que para ello es necesario identificar los genes que codifican para los caracteres de importancia y desarrollar marcadores para estos genes. En el caso de *P. chichipe*, las características de interés son las que aparentemente han sido afectadas por la selección artificial, como las detectadas en el estudio de variación morfológica (Carmona y Casas en prensa).

La evolución de *P. chichipe* bajo domesticación (a manera de conclusión)

P. chichipe es una de las especies de cactáceas columnares de mayor importancia económica del Valle de Tehuacán-Cuicatlán, región en la que los estudios arqueológicos (Callen 1967; MacNeish 1967; Smith 1967) han mostrado que las cactáceas columnares han sido usadas por la gente desde hace alrededor de 12.000 años. Como se mencionó, esta especie se encuentra

actualmente bajo tres formas de manejo humano, i) poblaciones silvestres, ii) poblaciones con manejo silvícola *in situ* y iii) poblaciones cultivadas (Carmona y Casas en prensa), y estas formas de manejo implican diferentes grados de selección artificial que han dado lugar a diferencias morfológicas, fenológicas, fisiológicas y del sistema de apareamiento entre las poblaciones bajo diferente manejo.

El fruto es la unidad de selección más importante de la especie, y la diferenciación morfológica ha sido apoyada principalmente por diferencias en caracteres del fruto. Se han encontrado frutos más grandes, con mayor cantidad de pulpa, semillas más numerosas y pesadas, menor número de espinas por cm² y cáscara más delgada en individuos de poblaciones manipuladas por el hombre con respecto a individuos de poblaciones silvestres (Carmona y Casas en prensa). Cambios fisiológicos en cuanto a la germinación de las semillas fueron estudiados bajo condiciones homogéneas y se encontró una germinación más rápida y una tasa de germinación mayor en poblaciones manipuladas, tanto cultivadas como bajo manejo silvícola, que en poblaciones silvestres (Otero-Arnaiz et al. 2003). Aparentemente, estos cambios en morfología y fisiología asociados al proceso de domesticación se han mantenido debido a la existencia de aislamientos reproductivos parciales. Al parecer existe un aislamiento espacial parcial en donde los individuos y poblaciones más cercanos espacialmente son también los más similares genéticamente. Adicionalmente, el estudio del flujo génico indica que la mayoría de los apareamientos se llevan a cabo en un área no mayor a 40 m, lo que coincide con las observaciones preliminares del forrajeo de los visitantes florales. Esto crea una barrera espacial al intercambio de polen entre poblaciones, pero ésta es parcial, ya que alrededor del 30% del flujo de polen es externo a la población de referencia; esto da lugar a que las poblaciones vecinas estén intercambiando genes, que

las homogenizan aún cuando estas se encuentren bajo diferente manejo. También se encontró un aislamiento temporal parcial dado por un desfase en la floración entre poblaciones con diferente manejo. Adicionalmente parece ser que la gente ha seleccionado individuos capaces de autopolinizarse, por la ventaja que estos presentan en cuanto a la producción de frutos aún en condiciones de déficit de polen, lo cual se refleja en una mayor proporción de individuos autocompatibles en las poblaciones manipuladas que en las poblaciones silvestres. Esto contribuye parcialmente al aislamiento reproductivo entre poblaciones con diferente tipo de manejo.

En suma, el manejo de *P. chichi* por las comunidades indígenas del Valle de Tehuacán-Cuicatlán ha determinado cambios en caracteres morfológicos, fisiológicos, fenológicos y en el sistema reproductivo asociados al proceso de domesticación, tanto en poblaciones cultivadas como en poblaciones bajo manejo silvícola. Sin embargo el efecto del manejo no se ha reflejado en reducciones de la variación genética neutral debido a que el entrecruzamiento es elevado y el flujo génico entre individuos y poblaciones cercanas ha homogenizado la variación entre poblaciones bajo diferentes tipos de manejo.

La información morfológica, ecofisiológica, genética y de biología reproductiva que sobre *P. chichi* han generado diferentes estudios permite concluir que el proceso de domesticación en esta especie se encuentra en fases incipientes. El proceso de selección artificial a favor de los fenotipos con mejores características utilitarias opera tanto bajo manejo silvícola como bajo cultivo. Esta selección ha determinado cambios en la frecuencia de fenotipos con respecto a las poblaciones silvestres, en general aumentando la frecuencia de los mejores fenotipos en las poblaciones manipuladas. Pero el proceso selectivo también se expresa en diferencias en los patrones reproductivos, pues al parecer ha

determinado un aumento en la frecuencia de individuos que se autopolinizan y ha favorecido individuos cuya fenología determina una mayor disponibilidad temporal de frutos. También ha favorecido que las semillas de los individuos manipulados presenten un patrón de germinación más rápido y en mayor porcentaje. No obstante estas diferencias fenotípicas significativas, la diferenciación genética utilizando marcadores neutrales, aún cuando es significativa, no es tan marcada y quizá el análisis con marcadores cuantitativos permitirá observar otro patrón.

La diferenciación observada a distintos niveles, permite corroborar que la selección artificial tiene una intervención significativa tanto bajo cultivo como bajo manejo *in situ*. El que haya una influencia significativa bajo manejo *in situ* tiene particular relevancia pues permite apoyar la hipótesis de que en Mesoamérica el manejo silvícola pudo y puede involucrar procesos de domesticación y pudo tener un papel importante en el origen de la agricultura en la región.

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PRIMER NOTE

Isolation and characterization of microsatellites in the columnar cactus: *Polaskia chichipe* and cross-species amplification within the Tribe *Pachycereeae* (Cactaceae)A. OTERO-ARNAIZ¹, J. CRUSE-SANDERS², A. CASAS¹ AND J.L. HAMRICK³¹Instituto de Ecología, UNAM, Campus Morelia. Apdo. Postal 27-3 (Xangari) Morelia 58089, Michoacán, México. ²Rancho Santa Ana Botanic Garden, 1500N. College Ave., Claremont, CA 91711. ³Department of Plant Biology and Genetics, 2502 Plant Sciences, University of Georgia, Athens, Georgia 30602, USA**Abstract**

Microsatellite markers were developed for the columnar cactus *Polaskia chichipe* from central Mexico. After an enrichment procedure and three screening steps 87% of colonies contained microsatellites. A pair of primers for 10 loci (7 polymorphic) were developed, tested and used to estimate variation in samples of 18 to 45 individuals from the Tehuacan Valley, Mexico. Alleles per locus ranged from 2 to 8 (mean 5.28; sd 2.5). Range of expected heterozygosity values was 0.188-0.797 (mean 0.502; sd 0.25). These loci are particularly useful for more precise evolutionary studies, such as gene flow and breeding systems, for this cactus species.

Keywords: genetic structure, cactus, molecular markers, dinucleotide repeats, PCR, SSR

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Cactaceae includes approximately 1,600 species native to the Western Hemisphere where they are dominant in arid and semiarid zones (Nobel 2002), and have both ecological and economic importance (Casas & Barbera 2002). There are few reports on the genetic composition of natural cacti populations in the literature. Most are estimates of allozyme variation in columnar cacti populations pollinated by bats (Parker & Hamrick 1992; Neel *et al.* 1996; Sahley 1996), and almost nothing is known about the genetic diversity of cacti with other gene dispersal mechanisms and breeding systems (Nassar *et al.* 2002). Species studied have high levels of genetic variation and low differentiation among populations (Hamrick *et al.* 2002). Microsatellite markers have not been developed previously for cactus species, but are essential for detailed evolutionary studies.

Evolutionary trends in reproductive biology of *Polaskia chihipe* have been previously investigated (Otero-Arnaiz *et al.* 2003). Presently our research

focuses on analyzing genetic structure and gene flow among populations in relation to the human management of populations. Here we report the characterization of seven polymorphic microsatellite loci isolated from *P. chichipe*.

DNA was extracted from flower buds following a protocol (de la Cruz *et al.* 1997) adjusting amounts for extraction in tubes of 1.5 ml. A genomic library was made and enriched for the motif CA/GT following the procedure by (Kandpal *et al.* 1994). Total DNA was digested with *Sau3A*I, and fragments between 400 and 1500bp were selected, purified and ligated with T₄ to *Sau3A*. Fragments were amplified using *Sau*-L-A as primer, PCR product was denatured and enriched by hybridization for the repetition (CA) using the matrix VECTREX® Avidin-D. Molecules recovered from the hybridization were amplified with *Sau*-L-A primer and the product purified. Cloning was conducted using the vector TOPO-TA cloning kit for sequencing (Invitrogen), and transformation in TOP10 One-shot chemically competent *E. coli* (Invitrogen), according to the manufacturer's protocol.

Table 1. Characteristics of 7 microsatellite loci identified in *Polaskia chichipe*. Sequences of the primers, annealing temperature and size of the cloned allele are showed. The number of individuals genotyped (n), alleles (A), observed and expected heterozygosities (H_O and H_E respectively) are indicated

Locus	SSR sequence in	PCR primer sequence (5'-3')	T_a	Size	n	A	H_O/H_E
Genbank no.	library			(bp)			
<i>Pchi5</i>	(CAC) ₉ ACA	CAAGAAAAAGGAAGCCCAACA	60	221	44	3	0.114/ 0.188*
AY147840		GGTGCCATATCCCTGTGTA					
<i>Pchi9</i>	(CT) ₁₆ (GT) ₆ AT	GTGGCCGAGAAAGAAGTTTG	60	211	41	4	0.415/ 0.397
AY147838	(GT) ₄ TT(GT) ₁₃	AAAGGCCCAAATCATAAGCA					
<i>Pchi21</i>	(CT) ₅ (AT) ₃ (GT) ₈	CGTTTAGCCCCTCTTTCTCC	60	124	41	6	0.634/ 0.772*
AY147837	GA(GT) ₅	GTTCCCAACTGACCGACAAC					
<i>Pchi25</i>	T ₅ (GT) ₁₆ A ₅	GCCCTTCTAAGGCCATTCT	60	273	18	5	0.353/ 0.715*
AY147836		ATCCGTGTCAAGATTGTGC					
<i>Pchi44</i>	(CA) ₁₇	ATTCAAACAGGCCACACAG	59	137	45	4	0.244/ 0.225
AY147834		GGGTGTTAGAAGGAATAATAGCTTG					
<i>Pchi47</i>	(TG) ₁₅	GTCCTGTGGCTAGCCCTTT	60	120	28	2	0.428/ 0.415
AY147832		CCATTCTCTCGCCATCTG					
<i>Pchi54</i>	(CA) ₅ CG(CA) ₅	CCTTGAGCTTTGACATTGAGA	60	170	30	8	0.733/ 0.797*
AY147831	TG(CA) ₂₂ (TA) ₃	GGAAGTTTTTCATTGGATGAG					

• Significant heterozygote deficiency ($P < 0.05$)

Colonies were hybridized with (CA)_n probe and hundreds of positive clones detected with chemiluminescence. After three screening steps, 23 positive clones were isolated and purified. Plasmids were sequenced with the Big Dye terminator kit (Applied Biosystems) using T7 and M13 universal primers, and detected with an ABI-PRISM 3700 DNA sequencer. Primers were designed using the Primer3 program (Rozen & Skaletsky 1998) to amplify segments from 100 to 300 base pairs in length for 20 sequences containing useful microsatellite repeats. Unlabelled primers were tested for amplification effectiveness in six individuals of *P. chichipe* and 2% agarose gels were used to separate fragments.

PCR reactions were performed using 15µl reactions: 100ng DNA, 0.25mM each dNTP, 2.5mM

MgCl₂, 0.5µM of each primer, 0.5 units of *Taq* DNA polymerase (Perkin-Elmer), and 1x *Taq* buffer (10 mM of TrisHCl, pH 8.3; 50 mM KCl; 0.001% w/v gelatin, Perkin-Elmer). Reactions were denatured at 94°C for 4 min, followed by 25 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min and a final extension at 72°C for 4 min, using a GenAmp 9700 thermo-cycler (Applied Biosystems). Fluorescent-labeled reverse primers were purchased for 10 loci for which amplification was effective. Variability of microsatellite loci was determined with an ABI 377-96 DNA sequencer in GENESCAN mode to detect the labeled primers in a 4.5% denaturing polyacrylamide gel. Allele scoring was performed using GENOTYPER software.

Table 2. Transferability of *Polaskia chichipe* microsatellite primers to other species of columnar cactus and the origin of the samples.

Species	Origin	<i>Pchi5</i>	<i>Pchi9</i>	<i>Pchi21</i>	<i>Pchi25</i>	<i>Pchi46</i>	<i>Pchi47</i>	<i>Pchi54</i>
<i>Carnegieia gigantea</i>	Sonora	-	-	*	*	*	*	*
<i>Lophocereus schottii</i>	Sonora	*	*	*	*	*	*	*
<i>Pachycereus pringeli</i>	Sonora	*	-	-	*	-	-	-
<i>Pilosocereus lanuginosus</i>	Venezuela	-	-	-	-	-	-	-
<i>Subpilocereus repandus</i>	Venezuela	*	-	*	*	-	-	-
<i>Stenocereus thurberii</i>	Sonora	-	*	*	*	-	-	*
<i>S. stellatus</i>	Tehuacan Valley	-	*	*	-	-	-	-

*indicate amplification of a homologous PCR product.

- indicate no amplification.

Two primer pairs gave no PCR product in the expected size range, the remaining eight gave high quality fluorescent PCR products that were within the size range of the cloned sequence, and one of them was monomorphic. Allele number in plants from the studied locality ranged from 2 to 8, and expected heterozygosities from 0.188 to 0.797 (Table 1). No linkage disequilibrium was detected among these loci ($P > 0.05$). *P. chichipe* exhibit significant multiloci heterozygous deficit ($P = 0.0001$, U-Test). This could be because the mating system with self compatibility and matting among relatives and also the low observed heterozygosities at *Pchi5* and *Pchi25* suggest that null alleles may be present at these loci. All analyses were performed with GENEPOP (Raymon & Rousset 1995)

These markers will be used to describe the genetic structure and estimate gene flow among populations of *P. chichipe* with different types of management (wild, silvicultural management and cultivated) in the Tehuacan Valley.

In order to test cross-species amplification of a homologous product in seven columnar cactus of the Tribe *Pachycereeae*, DNA was extracted from two individuals per species and PCR conducted as above. Results of the test for cross-species amplification are summarized in Table 2. In six of the seven nontarget species tested at less two of the primer pairs showed clear amplification.

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