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FLUJO GÉNICO EN CALABAZA (Cucurbita SPP.) DENTRO DEL SISTEMA MILPA EN EL OCCIDENTE DE MEXICO

TESIS

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DEDICATORIA

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CONTENIDO

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RESUMEN GENERAL

Con el fin de conocer los niveles de flujo génico entre dos especies cultivadas de calabaza (*Cucurbita argyrosperma* ssp. *argyrosperma* y *C. moschata*) y su pariente silvestre, *C. argyrosperma* ssp. *sororia* que se desarrolla cerca de las parcelas de cultivo, y su influencia sobre la diversidad genética de estos tres taxa de *Cucurbita*, se realizó este estudio en el SO del estado de Jalisco, México, en donde la calabaza se cultiva asociada al cultivo principal, el maíz. Las plantas de *Cucurbita* son monóicas, no tienen apomixis y requieren de polinizadores externos, lo cual posiblemente facilita el flujo génico entre taxa. En la primera parte del estudio, por medio de entrevistas directas a los productores en tres municipios de la región, se registraron las prácticas de selección y manejo del cultivo, con el fin de entender cómo podrían afectar éstas a la diversidad genética de las poblaciones de calabazas. Los agricultores manifestaron estar conscientes de la posibilidad de hibridación y flujo génico entre las calabazas cultivadas y entre ellas con el taxón silvestre. Los campesinos en general seleccionan pocos frutos (5 - 20) para la siembra del siguiente ciclo, de acuerdo con el tamaño de la parcela. En la selección de frutos para la siembra consideran las características típicas de cada variedad local que utiliza cada agricultor; esto ayuda a mantener la identidad propia de sus cultivares locales, aún frente al intenso flujo génico que se reporta. El intercambio de semillas para la siembra se ha llevado a cabo por un gran número de productores (70%) en diferentes intervalos de tiempo, ya sean anuales o más ocasionales. El 50% del total de los agricultores entrevistados han dejado de sembrar calabaza, y dos terceras partes de éstos han dejado de cultivarlas en los últimos seis años. Esto puede determinar una rápida pérdida de la base genética de este cultivo. Por todo lo anterior, al parecer los agricultores por medio de sus prácticas culturales inciden de manera significativa sobre la diversidad genética de las calabazas. En la segunda parte del trabajo, se exploraron métodos directos de estimación del flujo génico en las calabazas. El ciclo de apertura diario de las flores no mostró diferencias significativas entre taxa. La presencia de abejas polinizadoras mostraron regularidad en número y tiempo de visitas en los tres taxa de *Cucurbita* estudiados. Se usaron polvos fluorescentes como análogos del polen, los cuales fueron movidos por las abejas entre los tres taxa, permaneciendo el 50 % del polen en cada taxón, mientras que el resto se distribuyó hacia los otros dos taxa de *Cucurbita*. En una tercera fase se estudió la variación genética de estas calabazas por medio de electroforesis de isoenzimas, para inferir el papel del flujo génico en su estructura genética de las poblaciones. Se analizaron 12 loci polimórficos y se calculó

la diversidad genética de 16 poblaciones de *Cucurbita*. Resultó una diversidad genética relativamente alta (heterocigosis esperada = 0.407), con poca diferenciación entre poblaciones de los tres taxa ($D = 0.081$; $F_{ST} = 0.087$; $N_em = 5.22$), lo cual sugiere niveles altos de flujo génico entre los tres taxa estudiados de *Cucurbita*, e indica que el flujo génico es importante para mantener la diversidad genética de las calabazas. A pesar de los altos niveles de flujo génico encontrados, los agricultores mantienen la identidad de cada taxón, ya que seleccionan sus variantes propias. Al reducir la superficie y el número de agricultores que siembran calabaza, se disminuirá la diversidad hasta ahora presente. Las condiciones en general dentro de la milpa son propicias para que se presente flujo génico entre las variedades transgénicas y variantes locales y silvestres de calabaza, por lo que debemos ser muy precavidos con el uso de estos materiales transformados genéticamente.

Capítulo I

INTRODUCCION

Este estudio forma parte del proyecto interinstitucional titulado "Conservation of Genetic Diversity and Improvement of Crop Production in Mexico: A Farmer-Based Approach", financiado por la Fundación Mcknight (USA), dirigido por el Instituto de Biología de la Universidad Nacional Autónoma de México y por la Universidad de California, en Davis (USA). En este trabajo se estudió en forma integral el sistema milpa (asociación maíz – frijol – calabaza - quelites) bajo tres objetivos principales: a) describir y analizar la relación entre el conocimiento del agricultor, factores socioeconómicos y la diversidad genética en el agroecosistema milpa, b) caracterizar la estructura de la biodiversidad de los cultivos y la magnitud del flujo de genes de las plantas silvestres o parientes en maíz, frijol y calabaza y c) desarrollar y evaluar métodos de mejoramiento participativo con los agricultores, para mejorar la productividad de germoplasma criollo a través de selección masal o introgresión de germoplasma mejorado o parientes silvestres.

En el presente trabajo se estudiaron los procesos que determinan el flujo y estructura génica de tres taxa de calabazas, dos cultivadas (*Cucurbita argyrosperma* ssp. *argyrosperma* y *C. moschata*) y un tipo silvestre (*C. argyrosperma* ssp. *sororia*), bajo el escenario de la milpa. La zona de estudio se ubicó en el SO del estado de Jalisco, en los municipios de Autlán, Ejutla, El Grullo y El Limón (Fig. 1). En esta región se cultivan las dos especies de calabazas referidas y se distribuye el pariente silvestre cercano de ambas calabazas, por lo que en esta zona se efectuaron los estudios sobre flujo génico. Un quinto municipio (Amacueca, Jal.) se incluyó en el estudio, pero únicamente en el análisis de diversidad genética, pues están ausentes las variantes silvestres. De este municipo se analizaron dos poblaciones de calabaza originarias de Tepec (Fig. 1), una de las cuales fue de la especie *C. pepo*, la cual se usó como grupo externo y otra fue una variante de *Cucurbita argyrosperma* ssp. *argyrosperma*.

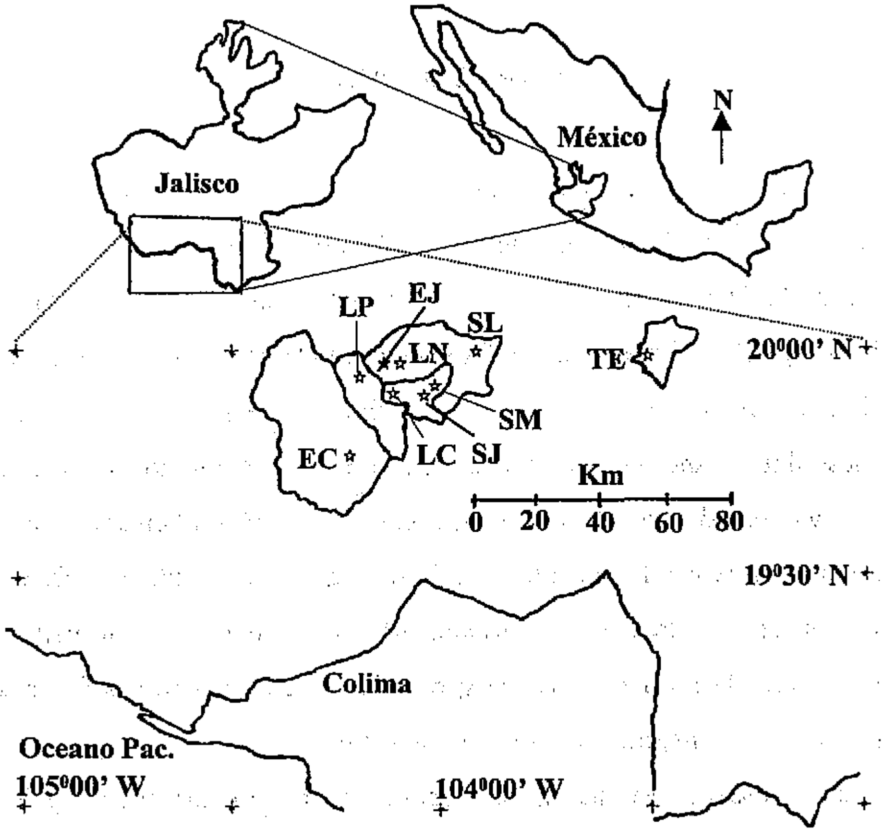

Fig. 1. Localidades en donde se realizó el estudio. EC = El Chante, Mpio. de Autlán, EJ = Ejutla, Mpio. de Ejutla, LC = La Cienega, Mpio. de El Limón, LN = Los Naranjos, Mpio. de Ejutla, LP = Los Parajitos, Mpio. de El Grullo, SJ = San Juan de Amula, Mpio. de El Limón, SL = San Lorenzo, Mpio. de Ejutla, SM = San Miguel, Mpio. de El Limón, TE = Tepec, Mpio. de Amacueca.

Las preguntas que se trataron de responder con el estudio fueron:

a) ¿Qué actividades realizan los agricultores para mantener la diversidad genética en las calabazas cultivadas (*Cucurbita argyrosperma* ssp. *argyrosperma* y *C. moschata*)?

b) ¿Cómo perciben los agricultores el flujo génico entre las calabazas cultivadas y entre éstas y el taxón silvestre (*C. argyrosperma* ssp. *sororia*)?

c) ¿Cuáles son las condiciones existentes del sistema de milpa, que favorecen o limitan el flujo génico entre las especies cultivadas y entre ellas y el taxón silvestre?
d) ¿Cómo influyen la fenología y la ecología reproductiva de las plantas de calabaza en el flujo génico dentro y entre los taxa analizadas?
e) ¿Es afectado el movimiento del polen por el número, tiempo y preferencias en las visitas de los polinizadores de las calabazas?
f) ¿Existe flujo génico entre los tres taxa de calabaza estudiados?
g) ¿Cuánta diversidad genética existe dentro de las poblaciones de calabaza de cada taxa?
h) ¿Cómo está distribuida esta diversidad genética entre las poblaciones de calabaza?

Las hipótesis que se plantearon en el estudio fueron:

a) Las actividades relativas a la forma de escoger la semilla para siembra y su intercambio entre agricultores afecta la diversidad de las calabazas cultivadas.
b) La utilización de diversas tecnologías pueden influir en la reducción de la superfcie de siembra de las calabazas.
c) La fenología de floración de los tres taxa de *Cucurbita* favorece la introgresión entre ellas.
d) Se carece de discriminación por parte de los polinizadores para las tres taxa de *Cucurbita*, lo cual favorece el flujo génico entre ellas.
e) Existe poca diferenciación genética entre las poblaciones de *Cucurbita*, debido al flujo génico entre los tres taxa de *Cucurbita*.

Antecedentes generales

México es un país de gran tradición agrícola, con diferentes condiciones ecológicas y culturales bajo las cuales se ha llevado a cabo la agricultura tradicional. Esta diversidad de habitats y culturas ha determinado el cultivo de diversas variantes locales con usos y generalmente con fenotipos específicos, propiciando la conservación de una amplia base genética de las especies cultivadas (Bye, 1993; Hernández, 1993). Además, en dichos agroecosistemas existe una evolución dinámica hacia nuevos cultivos, favorecida por la presencia de diversas razas o variantes de una misma especie (Hernández, 1973; 1993), incluyendo la presencia de sus parientes silvestres dentro y alrededor de los sistemas agrícolas (Harlan, 1976). Los parientes silvestres de las plantas cultivadas representan un sistema interesante desde el punto de vista agrícola y evolutivo, ya que estas plantas generalmente almacenan gran cantidad de variación genética (Doebley, 1992), lo cual podría ser de interés presente o futuro para los programas de mejoramiento genético (Kirkpatrick y Wilson, 1988; Doebley, 1990a), además de proporcionar información sobre su proceso de domesticación (Harlan, 1975; Hawkes, 1983; Doebley, 1990b).

El sistema agrícola tradicional conocido como "milpa", en donde se cultivan en forma asociada maíz - frijol - calabaza o bien, maíz - calabaza, representa un interesante escenario para el estudio de la diversidad genética de los cultivos participantes, por ser un sistema agrícola en donde generalmente se utilizan variantes locales. Para el caso de las calabazas, es muy común que se siembren dos y en algunos casos hasta tres especies juntas. Además, en algunas zonas del país se encuentran parientes silvestres de este cultivo que crecen en forma espontánea y natural junto a las plantas cultivadas, de tal modo que es muy posible que se realice cruzamiento natural entre los tipos silvestres y las calabazas cultivadas (Wilson, Lira y Rodríguez, 1994; Lira, 1995).

Historia y origen de las calabazas cultivadas

El género *Cucurbita* incluye a las calabacitas, calabazas y chilacayotes, como se les conocen en términos generales en México, y a los ayotes y zapallos en Centroamérica y Sudamérica, respectivamente. Es un género de origen Americano, tal y como lo demuestran los hallazgos arqueológicos en Perú y México (Whitaker y Bird, 1949; Whitaker, Cutler y MacNeish, 1957), y fue desconocido en el Viejo Mundo hasta el viaje de Colón a América en 1492 (Whitaker y Davis, 1962). Este género cuenta con aproximadamente 15 especies (Nee, 1990; Lira, 1995; Merrick, 1995); cinco de las cuales fueron domesticadas hace menos de 10,000 años, y se les consideran de las primeras plantas cultivadas de América, (Whitaker y Bohn, 1950; Whitaker y Cutler, 1965; Merrick, 1995; Smith, 1997; Piperno, Andres y Stothert, 2000).

De los taxa de calabaza cultivados, cada uno tiene un origen geográfico distinto. De acuerdo con Nee (1990) *Cucurbita maxima* Duch. ex Lam. es originaria de la parte sur de Sudamérica. *Cucurbita ficifolia* Bouché es quizá nativa de la parte central de América del Sur. Para *C. pepo* L. Nee (1990) sugiere como centro de origen la parte norte de México, para *C. argyrosperma* Huber. la parte sur de México y para *C. moschata* (Duch. ex Lam.) Duch. ex Poir. la parte sur de América Central y parte norte de Sudamérica (Nee, 1990). Aunque diversos taxa silvestres se han sugerido como posibles progenitores de las especies domesticadas de *Cucurbita*, el origen y las relaciones evolutivas entre muchas de estas especies han permanecido inciertos (Whitaker y Bemis, 1965; Decker, 1986; Andres, 1990; Decker-Walters et al., 1990; Nee, 1990; Merrick, 1995).

Importancia económica del genero Cucurbita.

El género *Cucurbita* es importante en los sistemas agrícolas locales de México y Latinoamérica, pero generalmente como cultivos secundarios o de un nivel menos importante con respecto a otros que son considerados como básicos en la alimentación humana, como es el caso del maíz, trigo, arroz, sorgo, etc. Sin embargo, las calabazas son ampliamente usadas en la alimentación humana mexicana, así como en muchas otras áreas del mundo (Whitaker y Bohn, 1950; Whitaker y Davis, 1962). En México se encuentran en forma común cuatro especies cultivadas de *Cucurbita*: *C. pepo*, *C. argyrosperma*, *C. moschata* y *C. ficifolia*, con un gran número de variantes locales que muestran la diversidad de cada una de ellas (Lira y Montes, 1992; Lira, 1995) y con ligera presencia de *C. maxima* (Sagarpa, 2001). Su uso se registra desde tiempos precolombinos hasta nuestros días (Whitaker y Bohn, 1950; Cruces, 1987) y desempeña un papel muy importante en varios sistemas de cultivo. En la producción de calabazas, un sistema muy importante es en el que la planta se produce en forma intensiva y su uso es como hortaliza fresca (calabacita); y otro es dentro del sistema tradicional conocido como milpa.

Respecto a la importancia económica de *Cucurbita* en México, para 1999 se registró una área cosechada de 28,675 ha de calabaza tierna (calabacita), 4,566 ha de calabaza madura y 11,309 ha de calabaza para obtener semilla, las cuales produjeron un total de 419,656, 43,760 y 5,794 toneladas de esos productos, respectivamente (Sagarpa, 2001). Sin embargo, existe una gran producción que no se registra, por ser su destino el autoconsumo (Montes, 1991). Por otro lado, *C. maxima* sólo se encuentra en forma natural en América del Sur, aunque en los últimos años se ha incrementado la superficie sembrada en México con variedades mejoradas introducidas, a las que se les denomina en forma general como Kabocha. Para 1999 se reportó una producción de 30,700 ton. de *C. maxima*, en una superficie cosechada de 1,347 ha (Sagarpa, 2001).

En las regiones de México en donde se utiliza la milpa y la calabaza está presente, el agricultor dispone de una amplia variabilidad morfológica (y presumiblemente genética) en este cultivo, la cual se manifiesta principalmente en forma, tamaño y coloración del fruto, cantidad de semillas producidas, calidad y grosor de la pulpa, tolerancia a enfermedades, precocidad en maduración del fruto, etc. (Whitaker y Davis, 1962; Lira y Montes, 1992; Lira, 1995).

Uso y valor alimenticio de las calabazas.

El uso de las calabazas en la dieta de la población americana se inició desde hace 8900 años (Smith, 1997), lo cual se ha demostrado por estudios arqueológicos. En diversas excavaciones se han encontrado vestigios de la utilización de estas plantas como alimento (Whitaker y Bohn, 1950; Whitaker, Cutler y MacNeish, 1957; Smith, 1997). Actualmente su participación en la dieta de la población incluye las partes vegetativas tiernas, la flor, los frutos tiernos y maduros y las semillas (Cruces, 1987; Lira y Montes, 1994; Lira, 1995).

En México se consume el fruto inmaduro como verdura, mientras que el fruto maduro se utiliza en la repostería típica. También sobresale el uso del fruto maduro como forraje, una vez que se le extrae la semilla. Las semillas, conocidas comúnmente como pepitas, se consumen en grandes cantidades, tostadas y saladas, como entremeses o bocadillos; además, son utilizadas como materia prima en la elaboración de moles, conocidos como "pipianes" o "pepianes" (Cruces, 1987; Montes, 1991; Lira y Montes, 1994; Lira, 1995). Por otro lado, de las pepitas se extrae aceite que sirve de base en la elaboración de jabones finos (Cruces, 1987). La flor masculina es también utilizada en la cocina mexicana para preparar diversos guisados, así como las "quesadillas de flor de calabaza", que son muy comunes en la parte central y sur del país; algunas partes vegetativas tiernas (hojas, zarcillos, primordios vegetales, entre otros) también son apreciadas como verdura (Cruces, 1987; Montes, 1991; Lira y Montes, 1992). Las semillas de las calabazas son el producto alimenticio y comercial más importante derivado de estos cultivos, principalmente por sus altos contenidos de aceites (> 43 %), proteínas (> 34 %) y fósforo (> 1 %). Por su parte, las partes vegetativas tiernas, las flores y los frutos tiernos y maduros, destacan por sus altos contenidos de calcio y fósforo, además de que las flores y frutos también son ricos en tiamina, riboflavina, niacina y ácido ascórbico (Esquinas-Alcazar y Gulick, 1983).

En relación con las propiedades medicinales que se le atribuyen a las calabazas, Hernández (1946) y Cruces (1987) mencionan que las semillas son útiles como vermífugo contra la solitaria (*Ascaris* sp. y *Tenia* sp.); la pulpa molida es usada contra irritaciones y quemaduras de la piel;

aplicándola sobre la parte enferma; además administrada acomo té, la pulpa de calabaza combate los cálculos renales.

Fenología y polinización de las calabazas.

Whitaker y Davis (1962) y Robinson y Decker-Walters (1997) indicaron que las especies cultivadas de *Cucurbita* son plantas monóicas, ya que presentan flores unisexuales en diferentes partes de la planta. Para que se realice su polinización es imprescindible la acción de insectos polinizadores, los cuales visitan una gran cantidad de flores, tanto estaminadas como pistiladas, para recolectar néctar y polen que las plantas de *Cucurbita* ofrecen en cantidades considerables para asegurar su polinización (Hurd, Linsley y Whitaker, 1971). Se han registrado diversas especies de abejas silvestres, de los géneros *Peponapis* Robertson y *Xenoglossa* Smith especialistas en su polinización (Hurd y Linsley, 1964; Canto-Aguilar y Parra-Tabla, 2000). Existe una asociación estrecha entre calabazas y abejas, tanto adultos como larvas se alimentan exclusivamente de néctar y polen de estas plantas, por lo que las abejas hembras han desarrollado pelos modificados en sus patas que usan para colectar y manipular grandes cantidades de granos de polen (Hurd, Linsley y Whitaker, 1971).

Las flores femeninas y masculinas de la calabaza abren únicamente un día, desde muy temprano en la mañana, exponiendo sus estigmas receptivos y anteras dehiscentes, esto es ofreciendo polen y néctar desde las primeras horas de la mañana, hora en que inician su actividad las abejas de ambos géneros. Además, existe una relación entre la distribución geográfica de las calabazas silvestres y cultivadas de América y las abejas de estos géneros (Hurd, Linsley y Whitaker, 1971). Al menos, dos especies de los generos *Peponapis* y *Xenoglossa* especializadas en la polinización de las calabazas se encuentran presentes en la zona de estudio de este trabajo (Ayala, 1988; Bautista, 1997). También es muy importante la participación de abejas generalistas, como *Apis mellifera* L. en la polinización de las calabazas, tal y como lo señalan Tepedino (1981), Avila et al. (1989), Bautista (1997) y Canto-Aguilar y Parra-Tabla (2000).

En la etapa de floración de las cucurbítas el ambiente juega un papel muy importante. Whitaker y Davis (1962) mencionan que los elementos del clima (temperatura y luz) influyen sobre la producción de flores masculinas y femeninas en diversas especies de *Cucurbita*. En los días largos con altas temperaturas se producen mayor número de flores masculinas, mientras que en los días cortos, con bajas temperaturas se induce la formación de flores femeninas en mayor proporción

(Robinson y Decker-Walters, 1997). Al respecto, Nepi y Pacini (1993) refieren que la hora de inicio de la floración, tanto en flores masculinas como en femeninas de *C. pepo*, varía dependiendo de la época del año en que se desarrollan las plantas, ya que en fechas con días mas largos (mayo-junio) los machos inician la apertura floral 15 minutos antes que las hembras. En cambio, en fechas más tardías (agosto-septiembre) esta diferencia no es significativa. Además, Nepi y Pacini (1993) en *C. pepo* hacen referencia a la gran diferencia entre el número de flores masculinas y el de flores femeninas, encontrando una relación de 16.5:1. Por su parte, Delesalle y Buchmann (1991) estudiando una poblaciones de una cucurbita silvestre (*Apodanthera undulata* Gray), señalan que sí existe sincronización en el inicio de apertura entre las flores de diferente sexo. Estos autores encontraron una relación de 20:1, en número de flores estaminadas y pistiladas.

Por su parte, Scheerens et al. (1987) describen el desarrollo floral y patrones de crecimiento en una población de *C. foetidissima* HBK, encontrando similitud en el tiempo de apertura diaria de las flores de ambos sexos. Wilson, Lira y Rodríguez (1994) mencionan que las plantas de *C. argyrosperma* spp. *argyrosperma*, *C. moschata* (cultivadas) y *C. fraterna* L. H. Bailey (silvestre) presentan una fenología de floración muy similar, en relación al día y hora de apertura de las flores masculinas y femeninas. Este fenómeno propicia que se presente una polinización cruzada entre la especie silvestre y las cultivadas. Por otro lado, el translape de la fenología de floración de la subespecie cultivada *C. pepo* ssp. *pepo* y la silvestre *C. pepo* ssp. *texana* (Scheele) Filov, ha contribuido al cruzamiento natural entre ambas subespecies (Kirkpatrick y Wilson, 1988).

Distribución actual de las calabazas cultivadas.

Las calabazas cultivadas están actualmente distribuidas en todas las regiones tropicales, subtropicales y templadas del viejo y nuevo mundo. En general, las especies domesticadas presentan un patrón definido principalmente por la altitud. En México son cuatro las especies que se encuentran distribuidas ampliamente y que se presentan en los sistemas agrícolas tradicionales. Su distribución, es en forma general, la siguiente: *C. argyrosperma* y *C. moschata* se encuentran en lugares cálidos y en altitudes igual o menores de 1700 m sobre el nivel del mar (s.n.m); *C. pepo* crece en lugares a mayores altitudes desde los 1000 y hasta 2800 msnm y *C. ficifolia*, la cual se considera perenne, se localiza en altitudes mayores de 1300 m (Cutler y Whitaker, 1961; Whitaker, 1968; Lira, 1995; Merrick, 1995).

Bajo el sistema de milpa generalmente se siembran dos o más especies de calabaza juntas

(Lira, 1995; Bautista, 1997). La calabaza se siembra junto con el maíz y ambos se cultivan bajo diferentes sistemas de labranza del suelo: mecánica, tracción animal y manual; el uso de cada sistema depende principalmente de la orografía de las zonas agrícolas. Un implemento usado en la siembra manual es la coa, una pieza de hierro con punta, adherida en un extremo de una pieza larga de madera (cabo), la cual se usa en sitios inaccesibles a los implementos agrícolas de tracción animal y mecánica. A estas parcelas de cultivo se les denomina "coamiles". Pero incluso en lugares en donde puede usarse tracción animal, los agricultores prefieren usar la coa para la siembra, por el alto costo de los animales (bueyes, caballos o mulas), su manutención y la renta en caso de que no sean los dueños de ellos.

Plantas cultivadas y sus parientes silvestre en la zona de estudio

En el SO del estado de Jalisco existen parientes silvestres de diversas plantas cultivadas, entre ellas, de las integrantes del sistema de milpa, los cuales se desarrollan cerca o a veces dentro de las parcelas de cultivo. Así el maíz coexiste con el teosintle (*Zea mays* spp. *parviglumis* Iltis & Doebley), el frijol común con su forma silvestre (*Phaseolus vulgaris* L.). La coexistencia de formas silvestres y cultivadas en esta región se presenta en otros cultivos nativos, como el tomate verde o de cáscara (*Physalis philadelphica* Lam., cultivado y silvestre) y el jitomate cultivado (*Solanum lycopersicum* L.) coexiste con la forma silvestre [*Solanum lycopersicum* L. var. *ceraciforme* (Dunal) Spooner, Anderson y Jansen, comb. nov., antes *Lycopersicon esculentum* var. *ceraciforme* (Dunal) A. Gray] y la presencia del chile cultivado (*Capsicum annuum* L. var *annuum*). y de la forma silvestre (*Capsicum annuun* var *aviculare*).

Para el caso de las calabazas existe la forma silvestre, *Cucurbita argyrosperma* ssp. *sororia* (L.H. Bailey) Merrick y Bates, localmente denominada "ahuichichi" y "tololonche", la cual se distribuye desde el nivel del mar, hasta 1300 msnm (Merrick, 1991). En la zona de estudio se cultivan dos especies de calabaza, *C. argyrosperma* ssp. *argyrosperma* denominada "rayada", "patipona" o "puerquera" y *C. moschata,* conocida como "tamaloyota", o "de castilla", y es común que los agricultores tradicionales las siembren juntas en la misma parcela en asociación con el maíz (Bautista, 1997; Merrick, 1990).

Cruzamiento interespecífico en Cucurbita

Existen diversos reportes en la literatura sobre la posibilidad de cruzamientos interespecíficos dentro del género *Cucurbita*. Whitaker y Bohn (1950) muestran las diferentes posibilidades de cruzamiento entre todos sus miembros. Bemis y Whitaker en 1965, hacen referencia al cruzamiento de dos especies silvestres (*C. digitata* y *C. palmata*). Wilson (1990) y Lira (1995) hacen un recuento de la hibridación entre diferentes taxa de *Cucurbita*. Por su parte, Garzón, Montes y Becerra (1993) reportan la transferencia exitosa hacia cultivares comerciales de *C. pepo*, de la resistencia a varias enfermedades virales presente en algunas razas o variedades mexicanas de *C. moschata*, por medio de cruzamientos manuales, usando como puente una variedad de *C. argyrosperma*. Whitaker y Bemis (1965) y Merrick (1990; 1991) encontraro diferentes grados de compatibilidad en relación con la producción de progenie viable al realizar cruzamientos manuales entre diversas especies no domesticadas y las cinco cultivadas. Por ejemplo, la progenie fue viable al cruzar *C. argyrosperma* y *C. fraterna* por Wilson, Lira y Rodríguez (1994). Las posibilidades de hibridación entre los tres taxa estudiados, se sabe que son altas, ya que al realizar cruzamientos entre los miembros de las subespecies de *C. argyrosperma* se han reportado niveles altos de compatibilidad. *Cucurbita argyrosperma* y *C. moschata* se consideran dos especies hermanas o parientes muy cercanos (Wilson, 1990; Wilson, Doebley y Duvall, 1992). Por lo tanto, son altamente compatibles entre ellas, aunque hay reportes de que el nivel de compatibilidad disminuye cuando *C. moschata* es usada como progenitor femenino (Merrick, 1990; 1991).

Estimaciones del flujo génico en plantas

Para entender los procesos evolutivos de una especie es importante conocer la estructura genética de varias de sus poblaciones, la cual ha sido definida como la distribución espacial y temporal de alelos y genotipos en las poblaciones (Loveless y Hamrick, 1984). Esta estructura se manifiesta en diferentes niveles y patrones de variación genética, los cuales juegan un papel muy importante para determinar el potencial evolutivo de esa especie. Los patrones de variación genética están fuertemente determinados por el efecto de los cinco procesos evolutivos fundamentales: 1) deriva génica, 2) migración, 3) sistemas de apareamiento, 4) mutación y 5) selección natural. Es por esto que para entender la biología evolutiva de una especie es indispensable conocer su variación genética (Lewontin, 1974). Los estudios de variación genética involucran dos aspectos, el primero se refiere a la descripción de los niveles de variación mantenida dentro de las poblaciones; y el segundo concierne a la manera en como está repartida esa variación entre las poblaciones (Clegg y Brown, 1983).

Conocer la distribución de la variación genética en una especie nos ofrece información adicional para entender las interacciones entre características ecológicas y de historia de vida, tales como los mecanismos de polinización, dispersión de semillas y fecundidad, y de cómo estas interacciones moldean la estructura genética de las poblaciones (Hamrick, 1983). Los sistemas de apareamiento y el movimiento de genes se consideran los factores más importantes en la determinación de la estructura genética (Levin y Kerster, 1974; Clegg y Brown, 1983; Loveless y Hamrick, 1984).

El flujo de genes es la incorporación de genes al acervo genético de una población, procedentes de una o más poblaciones (Futuyma, 1998). Las poblaciones se diferencian dependiendo de la magnitud y direccionalidad del movimiento de genes. Los niveles altos de flujo génico aumentan el tamaño efectivo de las poblaciones y reducen la diferenciación local por deriva génica o selección natural. Por el contrario, si el flujo de genes es limitado, se reduce el tamaño efectivo de las poblaciones y aumenta la diferenciación entre subpoblaciones (Jain y Bradshaw, 1966; Endler, 1973; Hamrick, 1978; Ellstrand, 1992). Existen dos tipos de métodos para estimar el flujo génico en plantas: 1) métodos directos y 2) métodos indirectos.

1) Métodos directos. Ellos involucran observaciones detalladas de los movimientos de los gametos y propagulos de las especies bajo estudio, y tienen la ventaja de que proporcionan datos ecológicos sobre la dispersión y permiten saber bajo qué condiciones ecológicas opera el flujo génico. Tienen la desventaja de que el área geográfica y la escala de tiempo en la cual se puede observar la dispersión es generalmente una fracción pequeña de la amplitud de distribución geográfica de la especie, así como del tiempo en el cual se ha mantenido el contacto entre subpoblaciones (Kearns e Inouye, 1993). Estos métodos pueden sobrestimar el flujo génico, ya que los individuos o gametos inmigrantes no siempre tienen éxito en la reproducción dentro de su nueva población (Futuyma, 1998). Además, los individuos que se dispersan fuera de dicha área no son considerados y la dispersión a larga distancia puede ocurrir esporádicamente y no ser detectada por estudios de corto plazo (Ellstrand, 1992).

Existen diversas formas de estimar el flujo génico con métodos directos:

a) Movimiento de polen marcado. Su ventaja radica en que es la forma más directa de estimar el movimiento de gametos. Su principal desventaja es que los materiales "análogos" que se usan para simular el polen pueden viajar en forma distinta al polen. Este método ha sido usado en diferentes plantas (Rademaker y de Jong, 1998), en general con buenos resultados, y también ha sido usado en plantas cultivadas y sus parientes silvestres más cercanos, como el estudio en rábano (*Raphanus sativus* L.) por Ellstrand y Marshall (1985). En *Cucurbita* también han mostrado éxito (Ordway et al., 1987; Montes-Hernández, Domínguez y Eguiarte, s/f).

b) Marcaje de visitantes florales. Esta es otra forma de inferir la dispersión del polen en especies que son polinizadas por insectos u otros animales (Englund, 1993; Karron et al., 1995; Futuyma, 1998).

c) Marcaje de semillas. Tiene interesantes ventajas, ya que al seguirles la pista a todas las semillas de las plantas utilizadas como tratamientos de análisis, se puede detectar la presencia de un inmigrante en las población (Parra, Vargas y Eguiarte, 1993; Cain, 2000).

d) Marcadores morfológicos. Al utilizar un carácter morfólogico dominante, en la primera generación se puede estimar su presencia en la progenie. En el caso del maíz, este método es posible con el locus *xenia*, en donde cualquier color de grano es dominante sobre el grano blanco (Doebley, 1990a). En melón se ha utilizado un color dominante en los cotiledones (Handel, 1982).

e) Poblaciones híbridas (Hedrick, 2000). Por medio de marcadores morfológicos en las plantas cultivadas y sus parientes silvestres, es posible detectar poblaciones híbridas. El intercambio de genes entre ellas ha promovido la convergencia morfológica entre algunas poblaciones cultivadas y silvestres de diferentes plantas, en las que es posible distinguir fenotípicamente la presencia de hibridación intraespecifica, como en el caso del arroz (Langevin, Clay y Grace, 1990), la calabaza (Wilson, 1990), el frijol (Beebe et al., 1997), el rábano (Klinger y Ellstrand, 1994), la canola (Jorgensen y Andersen, 1994), el maíz (Wilkes, 1977), la vainilla (Nielsen, 2000) y la alfalfa (Jenczewski, Prosperi y Ronfort, 1999). Usando marcadores citogenéticos, la recombinación intraespecífica entre plantas cultivadas y silvestres hace posible que se presenten cambios en el cariotipo (por ejemplo, la presencia y ubicación de nudos cromosómicos), con lo que se ha podido evaluar la hibridación entre ellos, como en el caso de la canola (Jorgensen y Andersen, 1994), el trigo (Jiang, Friebe y Gill, 1994) y el maíz (Kato, 1984).

f) Uso de marcadores genéticos. Estos se pueden dividir en dos tipos: uno o pocos loci y multilocus (Devlin y Ellstrand, 1990). Los marcadores más usados en estos análisis han sido las isoenzimas. Algunos ejemplos recientes que han aplicado esta técnica son en el sorgo (Arriola y Ellstrand, 1996), en la calabaza (Kirkpatrick y Wilson, 1988), en el rábano (Goodell et al., 1997; Klinger y Ellstrand, 1994), en el girasol (Arias y Rieseberg, 1994), en el betabel (Bartsch et al., 1999) y en el jitomate (Rick y Holle, 1990). Alternativamente, para estimar directamente la magnitud del flujo de genes se pueden usar varios loci a través de un análisis de paternidad. Para estos estudios generalmente se utilizan marcadores más variables, como RFLP's y microsatelites. Un estudio reciente de este tipo es el del betabel (*Beta vulgaris* L.) por Desplanque et al. (1999).

2. Métodos indirectos. Estos métodos utilizan observaciones de las frecuencias alélicas de las especies, datos que permiten deducir qué cantidad de flujo génico ha ocurrido para producir los patrones espaciales observados. Estos métodos dependen de los modelos de genética de poblaciones que predicen qué patrones se observarían en las diferentes etapas de flujo génico (Slatkin, 1999). Tienen la ventaja de que las estimaciones de flujo génico de las tasas promedio de dispersión es de muchos año y no solamente de un periodo en particular. También pueden detectar tanto la migración a larga distancia, como episodios raros y masivos de flujo génico, y fenómenos poco comunes de extinción y recolonización (Slatkin, 1985a). Además, se puede estimar el número promedio de inmigrantes intercambiados entre poblaciones locales, tomando como base la distribución de frecuencias de alelos raros o exclusivos (Slatkin, 1985b).

Entre los estadísticos indirectos propuestos para estimar flujo génico, se encuentran la F_{ST} de Wright (1951) y sus equivalentes, la G_{ST} (Nei, 1973), θ (Weir y Cockerham, 1984) y R_{ST} (Slatkin, 1995). La F_{ST} se calcula a partir de las frecuencias genotípicas de cada locus y mide la fijación relativa de alelos alternativos en diferentes subpoblaciones, comparando el promedio de las heterocigosidades de las subpoblaciones con la heterocigosidad total esperada bajo apareamiento al azar. La magnitud de la F_{ST}, por lo tanto, depende del nivel de divergencia entre las subpoblaciones en las frecuencias alélicas y se deriva de $F_{ST} = (H_T - H_S)/H_T$, donde H_S es la proporción promedio de heterócigos esperada en las subpoblaciones y H_T es la proporción de heterócigos promedio esperada a nivel global (Hartl y Clark, 1989; Neigel, 1997). Wright (1951), considerando neutralidad y equilibrio de alelos para cada locus, demostró que $F_{ST} = 1/[4N_em + 1]$. De este resultado se puede obtener una estimación de N_em a partir de la calculada con base en las frecuencias alélicas, $N_em = 1/4[1/F_{ST} - 1]$. Este método indirecto tiene la ventaja de incorporar todos los tipos de dispersión (eventos comunes e inusuales) y el promedio efectivo de la dispersión en el tiempo. El número de migrantes que ingresan a cada población por generación se denota como N_em, donde N_e es el tamaño efectivo de la población y m es la tasa de migración o la proporción de una población que migra por generación (Slatkin, 1987, 1993; Hartl y Clark, 1989; Neigel, 1997). La migración o flujo génico es un factor importante que puede afectar la composición genética de las poblaciones, al determinar el grado en que una población local es una unidad evolutiva independiente de otras poblaciones de la misma especie. Si N_em >4.0, el flujo génico es elevado y las poblaciones evolucionan conjuntamente, mientras que si N_em <<1.0, puede concluirse que hay poco flujo y cada población evoluciona de manera independiente (Hartl y Clark, 1989; Neigel, 1997; Hedrick, 2000).

Se pueden efectuar análisis adicionales para inferir el movimiento de genes, basados en relaciones espaciales sugeridas por modelos de aislamiento por distancia. Así, se pueden estimar las correlaciones entre distancia geográfica y distancia genética y a partir de ellas se puede describir la acumulación de diferencias genéticas locales bajo dispersión geográficamente restringida (Wright, 1951; Slatkin, 1993). Al respecto, Aguirre-Planter, Furnier y Eguiarte (2000) encontraron relaciones significativas entre flujo génico y distancia geográfica en *Abies religiosa* (Kunth) Schltdl. & Cham. Las poblaciones más cercanas se comportaron de manera casi panmítica entre ellas, mientras que las más lejanas eran muy diferentes.

Existen diferentes marcadores que se han utilizado en estudios de diferenciación genética y flujo génico entre plantas cultivadas y sus parientes silvestres (Ellstrand, Prentice y Hancock, 1999; Jarvis y Hodgkin, 1999). En una lista de estudios recientes se tienen trabajos con isoenzimas en *Cucurbita* (Kirkpatrick y Wilson, 1988; Wilson, 1990; Decker-Walters et al. 1990; Montes y Eguiarte, 2002), frijol (Beebe et al., 1997), algodón (Wendel, Brubaker y Percival, 1992), rábano (Klinger, Arriola y Ellstrand, 1992), canola (Jorgensen y Andersen, 1994), vainilla (Nielsen, 2000), alfalfa (Jenczewski, Prosperi y Ronfort, 1999), y maíz (Doebley, 1990a y 1990b). Otros marcadores, como RAPD's, se han usado en manzana (Durham y Korban, 1994), girasol (Linder et al., 1998), y canola (Jorgensen y Andersen, 1994); mientras que el análisis de ADN de cloroplasto se han usado en maíz (Doebley, 1990a y 1990b). Existen casos en los que se han combinado ambos tipos de métodos (directos e indirectos) en un mismo evento de evaluación, por ejemplo en canola (Jorgensen y Andersen, 1994) y en alfalfa (Jenczewski, Prosperi y Ronfort, 1999). En estos estudios se muestran las bondades de comparar y combinar los resultados.

Hibridación entre variantes cultivadas y sus parientes silvestres con variedades transgénicas.

En la última década se ha incrementado la atención a la posibilidad de hibridación y flujo génico entre plantas cultivadas y sus parientes silvestres, debido a que representa un camino potencial para el escape de transgenes a poblaciones naturales (Bartsch et al., 1999). De esta manera, los genes manipulados mediante ingeniería genética (transgénicos) en una planta cultivada pueden ser transferidos hacia sus parientes silvestres, y si ellos confieren una ventaja selectiva directa o indirectamente (como por interacciones epístaticas), podrían fomentar la formación de malezas más nocivas para la agricultura, al aumentar su adecuación. Por otro lado la hibridación de estas plantas modificadas con los parientes silvestres, les podría reducir a estos últimos los niveles de diversidad genética (Ellstrand y Hoffman, 1990; Kingler, Elam y Ellstrand, 1991; Raybould y Gray, 1993; Kingler y Ellstrand, 1994; Darmency, 1994; Dale y Irwin, 1995; Lefol et al., 1995; Hancock, Grumet y Hokanson, 1996; Ellstrand, 1997; Ellstrand, Prentice y Hancock, 1999; Pascher y Gollmann, 1999).

Con el uso de los diversos métodos de estimación de flujo génico antes expuestos, se reportan diversos estudios en los que se ha estimado la magnitud de este proceso entre miembros de una misma especie, pero considerando como una de las poblaciones a las plantas transgénicas. Entre otros estudios destacan los realizados en alfalfa (St. Amand, Skinner y Peaden, 2000), algodón (Llewellyn y Fitt, 1996), betabel (Bartsch y Pohl-Orf. 1996; Dietz-Pfeilstetter y Kirchner, 1998), canola (Pauk et al., 1995; Lavigne et al., 1998), melón (Hokanson, Hancock y Grumet, 1997), brocoli (Henzi, Christey y McNeil, 2000), calabaza (Spencer y Snow, 2001) y maíz (Quist y Chapela, 2001). Existen otros estudios en los que se ha evaluado el efecto de la hibridación intergenérica, como en el caso de la canola transgénica *Brassica napus* L. con *Hirschfeldia incana* (L.) Lagr.-Foss. (Lefol et al., 1995; Lefol, Fleury y Darmency, 1996) y la misma canola con *Raphanus raphanistrum* L. (Darmency, Lefol y Fleury, 1998). En la mayoría de estas evaluaciones se combinan ambos métodos (directos e indirectos), pero todos utilizan algún método molecular para confirmar la presencia del transgene en la F_1.

Estructura del estudio y objetivos

Esta tesis está dividida en cinco capítulos principales. El primero (el presente) es la introducción general, se plantea el sistema de estudio en general y los antecedentes de este grupo de plantas en el contexto particular del tema del flujo génico. En el siguiente capítulo se analizan los resultados del estudio sobre las prácticas que realizan los agricultores en relación con la conservación de la diversidad genética de las calabazas cultivadas y sobre la manera en la que perciben el flujo génico entre las calabazas cultivadas y su pariente silvestre. La información de ese capítulo se basa en los resultados de una encuesta directa (Anexo 1) aplicada a una muestra de 80 agricultores (Anexo 2) de tres municipios de la región del estudio. El tercer capítulo presenta observaciones sobre la historia natural de la calabaza y su relación con las oportunidades que se presentan para que pueda efectuarse flujo génico entre los tres taxa utilizados en la milpa. En el cuarto capítulo se presenta información sobre la estructura genética de las poblaciones de *Cucurbita* estudiadas, la cual se estimó con base en un estudio con isoenzimas (Anexo 3). Con base en esta información se hacen inferencias sobre los diferentes niveles del flujo génico. Por último, en el quinto capítulo se presenta la discusión y las conclusiones generales.

Los objetivos particulares de este trabajo fueron los siguientes:

a) Entender las prácticas culturales que realizan los agricultores, para mantener la diversidad genética de sus calabazas cultivadas (*Cucurbita argyrosperma* ssp. *argyrosperma*, *C. moschata*).

b) Determinar cómo perciben los agricultores el flujo génico entre las calabazas de este agroecosistema.

c) Evaluar si las condiciones existentes en la milpa son propicias para que pueda darse el flujo génico entre las especies cultivadas de calabaza y entre ellas y el taxón silvestre (*C. argyrosperma* ssp. *sororia*).

d) Conocer si la fenología y la ecología reproductiva de las plantas de calabaza influyen en el flujo génico entre los diferentes taxa de calabazas estudiadas.

e) Estudiar cómo las actividades de los polinizadores mueven el polen entre las calabazas estudiadas.

f) Investigar los patrones y niveles de flujo génico entre dos especies cultivadas y una silvestre que se desarrollan en el sistema milpa.

g) Describir la distribución de la variación genética usando isoenzimas, dentro y entre las poblaciones de calabaza estudiadas.

h) Estudiar las relaciones genéticas entre poblaciones en las tres taxa de calabaza.

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Capitulo II

Maintenance of squash (*Cucurbita* spp.) landrace diversity by farmers' activities in Mexico.

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Key words: squash, *Cucurbita*, landraces, diversity, farmers, Mexico.

Abstract

Squashes (*Cucurbita* spp.) are common components in traditional cropping systems in Mexico, mainly in the agroecosystem known as "milpa", in which squashes are cultivated in association with maize, the main crop. This study was carried out in three municipalities of the southwestern region of the state of Jalisco, Mexico, during the summer of 2000. Using a questionnaire, we surveyed 80 farmers on production and selection practices of their squash crops, in order to understand how these factors affect genetic diversity of local squash populations. We determined that the most of the farmers practicing squash cultivation were elderly (mean 60 years old). In the survey almost all farmers interviewed (97.5%) had grown squash but only half of them (50.0%) were still producing squash at the time of the study. The most common factors causing abandonment of squashes cultivation, according to farmers were use of maize herbicide and the current low price of maize. For sowing the next planting cycle, farmers generally select 5 – 20 fruits to obtain seeds. The numbers are markedly few compared with maize and beans from which were hundreds of fruits are used to obtain seeds for cultivation. Farmers recognize typical characteristics of each cultivated species and selection is directed to maintain their identity. Nearly two thirds of the farmers (62.0%) had exchanged seeds of squash for planting. This can increase the genetic basis of their squash germplasm. The wild gourd (*C. argyrosperma* ssp. *sororia*) has a widespread distribution in the region, especially as a weed close to or within crop fields. All of the surveyed farmers were conscious of the possible hybridization between it and their cultivated squashes. Farmers are able to

distinguish and eliminate this weedy squash and their hybrids.

Introduction

Squash (*Cucurbita* spp.) is one of the major crops domesticated in South Mexican and Central American center (Harlan, 1975; Merrick, 1995), which has spreaded throughout the world (Whitaker and Bohn, 1950; Cruces, 1987). It is cultivated for production of fresh vegetables (vegetable marrow, zucchini types) and mature fruit (cushaw, pumpkin types). In Mexico, this crop plays an important role in different agroecosystems, but the most common is being an integral component of a traditional crop system, known as "milpa", which consists of the association of maize-bean-squash or maize-squash (Lira, 1995). Both immature and mature fruits and seeds of *Cucurbita* species provide inexpensive sources of proteins and vitamins for diet of rural and urban families (Esquinas-Alcazar and Gulick, 1983). In addition, flowers and tender vegetative parts are also appreciated as fresh vegetables (Cruces, 1987; Lira, 1995).

In Mexico, four species of *Cucurbita* are commonly cultivated: *C. argyrosperma* Huber ssp. *argyrosperma*, *C. moschata* (Duch. ex. Lam.) Duch. ex. Poir., *C. pepo* L. and *C. ficifolia* Bouché. The first three species stand out as an important part in the economy and the feeding of Mexican population (Lira, 1995; Lira and Montes, 1992). In the last years in the NW of Mexico, some improved varieties of the species *C. maxima* Duch. ex Lam. have been introduced, which production is directed mainly towards export markets.

Since species of *Cucurbita* are no apomictic monoecious requiring the visits of specialist wild bees of genus *Peponapis* and *Xenoglossa* in order to set fruit (Hurd, Linsley and Whitaker, 1971; Bautista, 1997; Canto-Aguilar and Parra-Tabla, 2000); there are broad opportunities for inter- and intraspecific gene flow in *Cucurbita*.

Phenotypic diversity within landrace populations of *Cucurbita* - such as those found in milpa cropping systems in Mexico- is high, including variations in fruit form, size and color, number and size of seeds produced, quality, color and thickness of the fruit pulp, tolerance to pests, and precocity in fruit production, among other traits (Whitaker and Davis, 1962; Garzón, Montes y Becerra, 1993; Lira, 1995). In *Cucurbita* as well as in other crops, this variation is favored and maintained by selection for specific traits by farmers (Altieri and Merrick, 1987). Brush, Carney and Huaman (1981) mention that rural cultures in Peru classify and select their landraces according to a range of criteria, including agronomic, culinary, medicinal and/or ritual aspects. These criteria generate primarily two types of selective pressures: (1) selection to improve crop yield by selecting conspicuous traits, and (2) selective aimed to maintain landraces and characteristics within those populations that are important to the farmer for reasons other than productivity itself (Louette and Smale, 2000). Cultivation of these landraces according to Brush and Meng (1998), perpetuates local knowledge about crops and crop production, and this knowledge has served as an important resource for breeders and agricultural scientists in the formal sector.

Implementation of modern strategies to increase crop productivity, such as use of herbicides, and replacement of local traditional crops by others has caused reduction of genetic diversity of crops in areas with traditional agriculture (Altieri, 1991; Oldfield and Alcorn, 1987). Loss of genetic diversity may be more important in those areas where the crop originated and was domesticated (Brush, 1991; Bellon and Taylor, 1993), because levels of genetic variation within landrace populations in those regions commonly are high. In these areas, both wild relatives of cultivated plants and landraces in general have large amounts of genetic variation (Dobley, 1992), and for this reason they may be important sources of genetic diversity for cultivated plants and can be the basis for current or future crop improvement programs (Dobley, 1990; Wilson, 1990).

In western Mexico the ancestors of maize (*Z. mays* subsp. *parviglumis* Iltis & Doebley) and common beans (*Phaseolus vulgaris* L.) have survived until present, and they occur usually in small populations. In this same region two cultivated squash species, *C. argyrosperma* ssp. *argyrosperma* and *C. moschata*, and the wild type *C. argyrosperma* ssp. *sororia* (L.H. Bailey) Merrick and Bates grow in sympatry. There is experimental evidence of compatibility among these *Cucurbita* taxa (Merrick, 1990), and they are closely related phylogenetically (Wilson, Doebley and Duvall, 1992). High levels of gene flow between them have been detected in this region through population genetics studies (Montes and Eguiarte, 2002).

In order to understand the practices carried out by farmers to maintain genetic diversity of cultivated squashes, we conducted this study aimed at: 1) evaluating the cultural practices relevant to maintain genetic diversity of cultivated squashes, and 2) determining how farmers perceive and maintain distinct squash varieties despite the presence of gene flow. We test the hypothesis that the farmers' activities maintain the genetic diversity of cultivated squashes.

Materials and Methods

This study was conducted in three municipalities of the southwestern area of the state of Jalisco, Mexico (Table 1). In two municipalities we studied two localities, and one locality in other municipality. We used two sites because in the largest locality the number of maize farmers is low (less to 15), but in both cases, pairs of villages exhibit similar environmental conditions. The three municipalities were selected based on differences in environmental characteristics among them, as arable area, production systems, water resources for agricultural production, topography and crop types planted besides the maize crop (Martínez-Reding, 1992; INEGI, 2001) [Table 2]. In the three municipalities, a detailed survey was conducted to characterize the farmers' activities and criteria for maintaining squash diversity. Through formal personal interviews formal, farmers were asked questions and responses were discussed with them. Farmers were randomly selected for interviews in each municipality from a list of the Mexican government program called PROCAMPO (directed to support rural economy). The only requisite to select farmers was that they cultivated maize, because this support is only for maize crop. The total number of interviews was 80: 31 from Autlán, 29 from El Limón and 20 from Ejutla (Table 1). Before the formal survey, the questionnaire was tested with a sample of five randomly chosen farmers cultivating squash in El Chante. Questions were clustered into four themes: (1) seed selection practices for next cultivation season, (2) seed exchange practices among farmers, (3) the reasons why some farmers have stopped cultivating squash, and (4) local farmers' beliefs regarding gene flow.

Results

Data obtained from the survey shows that the two species of *Cucurbita* are cultivated in association with maize, the main crop in the study region. Sometimes only one *Cucurbita* species is cultivated, while in other cases both *Cucurbita* species together with maize. Only *C. argyrosperma* ssp. *argyrosperma* was cultivated with maize by 12.8% of the farmers (Table 3), and is referred to by a range of different local names: "rayada", "buchona", "patipona" or "puerquera". We did not find any correlation between the folk taxonomic names and the practical selection or uses (data not shown). *C. moschata* was sown alone with maize by 42.3% of all farmers (Table 3), it was more common in El Chante (Autlán), it is called "calabaza de castilla" and "tamalayota". The two species (*C.*

argyrosperma ssp. *argyrosperma* and *C. moschata*) were sown together in the same field by about half (44.9%, Table 3) of the farmers who were surveyed, with higher frequency in Ejutla (Table 3).

Most of the farmers (85.0%) that still cultivate maize associated with squash are elderly (> 50 years old) [Tables 4, and 5]. In all cases, farmers considered squash as secondary or tertiary in priority, after maize and another crops as vegetables, grass, etc., in spite of widespread squash use as human food in the form of immature and mature fruits, and seeds.

Only two maize farmers had never planted squash. One was from Autlán and other from El Limón. Half (50.0%) of the interviewed farmers had discontinued cultivation of squash sometime in the last 20 years (Tables 4, and 6). Out of those who had cultivated squashes it in the past but were no longer growing it nearly, two thirds (64.1%) had stopped producing squashes more than six years ago, while about one third (35.9%) had stopped in the last one or two years (Table 6). But those farmers that had stopped growing squashes, tended to be older (average 61.9 years old ± SD 12.46) than the farmers who were no longer engaged in squash production (average 57.8 years old ± SD 16.1). Among the younger farmers (≤ 50 years old), only about one third (31.6%) were still involved in squash production. In contrast, among older farmers (those over 50 years old), more than half (55.9%) were still producing squashes. There were some differences in these patterns among municipalities (Table 5). A slight majority (53.3-60.0%) of farmers in both Autlán and Ejutla were still engaged in squash production at the time of the study, whereas in El Limón it was less prevalent (39.3%). But when this practice was viewed in relation to the age of farmers, similar patterns were found between all three regions. In Ejutla and Autlán, older farmers were four times more likely to be growing squash than younger ones, and most of older farmers were still doing so. In El Limón, very few of the younger farmers were still growing squashes, whereas almost one third of the older farmers were doing so (Table 5).

In some cases farmers left squash production to concentrate only on maize production (Table 7). This was mainly due to the use of herbicides against weeds associated to maize, practice that was incompatible with production of squashes. Besides, when growing squash in association with maize, increases of general labor cost mostly by use of manual control of weeds. In other hand, low prices of squash seeds and fruits discourage its production (Table 8). Farmers who were not growing squash said that growth habit of squash vines causes the maize plants to lodge and, consequently, it is necessary to remove squash vines from maize stalks, thus increasing the amount of labor. In addition, presence of squashes in milpa restricts the use of agricultural machinery, hand weeding, and use of herbicides.

Other farmers have stopped cultivation of maize and, therefore, of squashes, mainly due the low prices of maize and the high cost of inputs such as herbicides, labor, and fertilizers, as well as the low productivity levels of maize. The proportion of farmers that have stopped planting both maize and squash is also high in El Limón (60.8%, Table 7). Almost one third of them produce maize in monoculture, and 10% of them grow a variety of maize selected for ear husks used in preparation of "tamales", which have high price. Additionally, one third of farmers in that village were sowing high value crops, such as melon (*Cucumis melo* L.), chili pepper (*Capsicum annuum* L.), or tomatoes (*Solanum lycopersicum* L. before *Lycopersicon esculentum* Miller). In Autlán half of the farmers had shifted from the traditional milpa cropping system to production of sugar cane (*Scharum officinale* L.) or mescal (*Agave angustifolia* Haw.), and in Ejutla only one farmer had bartered the milpa to planting grass for fodder.

Most farmers select carefully fruits for the subsequent growing season of squash cultivation. First they consider the typical characters of each species (Table 9). Seventy five percent of farmers mentioned the following set of traits which are selected for planting purposes: fruit size, form, weight, and health. The average number of fruits selected for planting varied, from 2 to 30 or more, with an average of 8.9 ± SD 12.3 (Table 10). This variation can be explained in part by the area destined to milpa production per household, which varied from one to seven hectares. Furthermore, the quantity of seeds used to plant *Cucurbita* is 0.5 kg/ha on average ± SD 0.3, which is relatively low when compared with the amount of seeds quantity that used for maize cultivation (20 kg /ha). The area for milpa production and number of fruits selected are not necessarily correlated (data not shown), since some farmers use a low number of seeds because their milpa plot is small (1 to 2 has), but they select a small number of seeds from different squash fruits, rather than selecting many seeds from one or few fruits. Only nine farmers (11.4 %) did not use fruits as units of selection. Instead, these farmers take a portion of the bulk seed, that would otherwise be used for consumption or sale (Table 10).

Seed selection is based exclusively on fruits previously selected. Farmers do not select seeds from plants in the field during the cropping season, but they choose the best fruits after harvesting. Before consumption of mature fruits by households as either food or fodder, the farmers review fruit phenotypes and may save seeds from the best phenotypes according to their criteria on quantity and quality of the product. Most of the farmers (77.9 %) considered large seed size and full, plump embryos as primary traits (Table 9). Almost all the farmers interviewed select both fruits and seeds by themselves (97.5%), rather than by involving people external to the household.

The amount of seeds that the farmers use for planting squashes per hectare was dependent in part on the distribution of squash plants within the maize plots (Table 11). If squash seeds are mixed with maize seeds, and both crop seeds are sown at same time, its distribution is at random, within the maize rows, but with higher quantity (≈ 50%) of squash seeds per ha on average. In contrast, the quantity of squash seeds was lower when only in a subset of maize rows some furrows of maize are intrercropped with squash. Under the latter planting design, squash seeds are typically sown in a maize row intercalated between some rows of maize alone (generally from five to ten), eight or ten days after maize sowing, when maize seedlings are present. Overall, nearly two thirds of the farmers (62.8%) mixed squash seeds with maize seeds before sowing (Table 11). However, this practice was observed to be especially common in Ejutla, where 90.0% of the farmers practice it. In contrast, about one third to over half of farmers in Autlán and El Limón, respectively, preferred to plant squash together with maize in some rows, separated by rows in which only maize is planted (Table 11). These differences in planting practices could be correlated with differences in topography. Most of the interviewees in Ejutla cultivated hillsides (Table 2), using the traditional method of slash and burn, in a crop system called "coamil". Under this system planting is generally manual, with aid of a "coa": an iron rod attached to a piece of wood, used to open a hole in the ground, where seeds of maize and squash are deposited. On other hand, in Autlán and El Limón most of crops lands are flat (Table 2), and farmers often use tractors for planting maize and subsequently sow a subset of their maize furrows with squash seeds by hand.

In relation to the exchange of seed stock among households, 35.9% of the farmers said that they have never exchanged seeds for sowing (Table 12), whereas 14.1% of farmers stated that it is necessary to exchange seeds, because if the same seed source population is used over time, yields decline. We did not observe differences among communities in these beliefs. Time interval for seed exchange was variable: 18.0% of the total number of farmers that exchanged seeds performed this practice every year, while 66.0% exchange seeds only occasionally (Table 13). Among those farmers who have exchanged seed stock of squash sporadically, the reason for the exchange was reported as principally due to two reasons: 1) to replenish seed supplies when the previous harvest netted low seed yield (19.8% of the farmers that have exchanged seed), and 2) to obtain sources of novel germplasm (39.7%). Out of the total number of farmers interviewed, 17.9% sowed seeds from a source that differed from their own seed stock during their last planting cycle involving squash production. Most of the seeds are exchanged among neighbors of the same town (86.4%), and the rest among other family members inside of the same villages (12.3%). For each species, the squash types that were recognized apparently present in those communities before have always been the same ones, since none of the interviewers reported loss of a particular variety that had originally been present in their community.

In relation to farmers' perceptions of the potential genetic interchange between *C. argyrosperma* and *C. moschata*, nearly half of the farmers (42.3%) believed that the two species are not able to hybridize spontaneously mainly because flowering phenology of the two species differs, with *C. argyrosperma* ssp. *argyrosperma* characteristically flowering considerably earlier than *C. moschata*. Nevertheless, 18.0% of the farmers said that they believe that it is possible that this phenomenon occurs, because there is a time period when staminate and pistillate flowers of both species occur simultaneously, and the same bee species visit both types of flowers. In addition, 18% of farmers noted that there is variation in the planting date for squash among farmers, and this variation makes possible the coincidence in flowering phenology of the two species. Some fruits show morphological characters intermediate between species or with characteristics of the other species; and there was a perception in this group (18%) of farmers that these fruits could be the product of interspecific hybridization. Nevertheless, those farmers reporting such observations indicated that the "intermediate" type fruits are not desirable for selection of seed stock for subsequent planting.

All of the farmers were aware of the presence of the wild/weedy squash *C. argyrosperma* ssp. *sororia* in their villages. Half of farmers (50.0%) have experimented the use of its fruit and seeds as medicine, and seeds for food. El Limón (60.7%) and Ejutla (55.0%) were the villages with higher participation in the use of this wild gourds. Most of farmers (93.6%) pointed out that it is possible that *C. argyrosperma* ssp. *sororia* crosses with the cultivated squashes in their fields. All of the farmers interviewed coincided in saying that the bitter flavor of some immature squash fruits - which they indicated occurred occasionally - is evidence of possible "mixture" among both cultivated and wild types squashes. Bitter flavor is characteristic of the wild types. All interviewed farmers reported that their wives taste the immature squashes before using them in food preparation. The farmers indicated that past experience has shown that only one bitter squash would render the food inedible. This behavior, to test squash fruits, is carried out with less frequency for mature squashes, because they use only one or two fruits for cooking, because the probability of finding it is low, instead the great number of immature fruit used for cooking. For controlling this weedy plant in the field it was reported by people that as soon as they recognize a wild plant within their squash plots they would remove it; also, farmers would eliminate such plants close to the milpas (mainly by cutting its branches). But they recognize that is difficult to eliminate all weedy plants in and around the milpa, because the few visits to the milpa for field maintenance.

Discussion

Most of farmers interviewed were elderly. Furthermore, most farmers said that they carry out activities of selection of squash fruits and seeds, because in all the families included in the survey, only few members were involved in the agricultural activities to cultivate squashes, and in general in all activities of the field, because there are few members in the family. The principal cause is that most young people migrate to the USA. We are deeply concerned about the knowledge of the traditional cultivation practice associated with these squash landraces could be lost among the younger farmers, because the they do not work and use these plants, as was previously described in the same general region of this study for several species (Benz et al., 2000).

In spite of the contribution of squash in diet of local farmers in this region, these plants occupie an insignificant place in the economy of the households of the farmers. Squash was reported to occupy on average the thirdth or fourth place among other agricultural commodities, after maize and other crops and cattle raising, because for most of the farmers it is not a "cash crop". However, squashes are still important items in Mexican popular culture. Several reasons are important to explain why people are stopping to grow squashes, including the use of herbicides, change of maize for other crops, mechanization of farming, among other causes. But among the most important is the low price of maize and an uncertain market for the products derived from squashes.

With regard to the possibility of intra- and interspecific hybridization, when both cultivated squash species occur in the same plot or within neighboring squash plots, the fact that there are several weeks in which both species are flowering simultaneously, and the same insects visit them (Bautista, 1997), along with the close relationship between them (Wilson, Doebley and Duval, 1992), suggest that hybridization occurs commonly.

Regarding the reduced mean number of fruits of squash that the farmers select in general for sowing and seeds that are used to sow per hectare, population size of squash is small, and this could represent a bottleneck, which could result in a serious loss in heterozygosity (Hedrick, 2000), and therefore, a loss of the *Cucurbita* variability. But the cultural practices, in particular the common practice of exchange of seeds, and the different methods for squash planting that may promote crossing among plots, probably decrease this potential problem. It is important that farmers select a part of the total phenotypic diversity of fruits of each variety, without consider just one ideal fruit form, fruit size, etc., but with an idea prestablished for each species of *Cucurbita.* Similar processes have been described in maize in a nearby region, where the farmers choose many maize ears, with a ideotype of landraces, but with a range of morphological traits that identify this local landrace own; there are marked difference with formal plant breeding where the variance of traits have to be very limited (Louette and Smale, 2000). Selection and maintenance of landraces are more clear in maize because number and definitions of landraces of maize are very large; in several regions of Mexico the farmers sow part of their maize plots with improved varieties, with obvious hybridization among landraces and improved maize varieties, but the farmers conserve their original materials of maize local varieties (Aguirre, Bellon and Smale, 2000; Louette, Charrier and Berthaud, 1997; Perales, 1998).

As we already explained, the exchange of seeds for planting among farmers could allow maintenance of the genetic diversity in these squashes populations and, consequently serve as a method by which farmers can increase the genetic basis of their squash germplasm. Use and benefits of increasing diversity by exchange of seed for planting has been reported in maize by Aguirre (1999) and Louette, Charrier and Berthaud (1997) in Mexico, and in local varieties of cowpea in Africa by Uguru (1998). The belief that the same seed stock should not be planted over successive seasons or that there is the need to "renew" it because its yield will decline has been reported for others crops and regions (Wood and Lenné, 1997; Louette and Smale, 2000). But in the central part of Mexico (States of Mexico, Morelos and Puebla) in a similar study, only 7.2% of the interviewed farmers had exchanged squash seeds for planting (Bautista, in preparation). In the case of our study all the above cultural practices explain in part the high levels of genetic variation at isozyme level and of gene flow found among these same taxa (Montes and Eguiarte, 2002).

The permanent contact between the two cultivated *Cucurbita* species and the wild relative, because they share common pollinators and the actions that carry out the farmers may be to maintain the phenotypic diversity of squash landraces (Lira, 1995; Lira and Montes, 1992). We have shown high levels of genetic diversity in these populations and extensive gene flow among these taxa (Montes and Eguiarte, in press). But we believe that because of the number of farmers that have stopped the cultivation of squash in the last 20 years, and more in the last six years, where two thirds of them have stopped planting squash, the elderly age of the squash farmers and the few number of seeds for sowing, the diversity of the squashes present could be in risk. We think that a scenery of dramatic genetic erosion in the near future in *Cucurbita* at the level of the region of this study is present. For all these reasons we consider that it is very important to promote an effort for a program of *ex situ* conservation of *Cucurbita* genetic resources as a germplasm bank.

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Table 1. Localities, numbers of interviewees, geographic position, and elevation (m) where the survey was conducted in the state of Jalisco, Mexico. Locality names that will be used throughout the text are Autlán = El Chante, El Limón = San Miguel Hidalgo and San Juan de Amula, and Ejutla = Ejutla and Los Naranjos.

Municipality	Locality	N / W	m	n
Autlán	El Chante	19^{0}43’09” / 104^{0}12’24”	919	31
El Limón	San Miguel Hidalgo	19^{0}51’07” / 104^{0}04’54”	804	19
El Limón	San Juan de Amula	19^{0}49’59” / 104^{0}04’42”	797	10
Ejutla	Ejutla	19^{0}53’56” / 104^{0}09’27”	1140	13
Ejutla	Los Naranjos	19^{0}54’59” / 104^{0}07’36”	1102	7

Table 2. Percentage of environmental and production aspects of the municipalities studied.

Attribute	Autlán	El Limón	Ejutla
Area of arable land %	61.5%	73.4%	20.6%
Area under irrigation %	21.5%	11.3%	3.5%
Rainfed area %	45.1%	60.2%	87.0%
Annual mean T^{0}C	23.5	24.8	22.8
Annual mean rain (mm)	997.5	887.3	878.7

Table 3. Percentage of farmers that had cultivated *Cucurbita* species in the municipalities studied.

Taxa	Autlán (n = 30)	El Limón (n = 28)	Ejutla (n = 20)
Only *C. argyrosperma* ssp. *argyrosperma*	3.3	39.3	75.0
Only *C. moschata*	66.7	35.7	10.0
Both *Cucurbita* species	30.0	25.0	15.0

Table 4. Percentage of squash farmers clustered into two age classes that were still engaged in squash production.

	Age class of farmers	
	≤ 50 years old (mean 38.4 ± 6.6)	> 50 years old (mean 66.3 ± 8.9)
Total that had grown squash at any time (n = 78)	24.4	75.6
Still growing squash at the time of the study (n = 39)	15.4	84.6
Stopped squash production before the study period (n = 39)	33.3	66.7

Table 5. Percentage of farmers still involved in squash production at the time of the study relative to the age of the farmer.

Age class of farmers	All farmers (n = 78)	Autlán (n = 30)	El Limón (n = 28)	Ejutla (n = 20)
≤ 50 years old	7.7	10.0	3.6	10.0
> 50 years old	42.3	43.3	35.7	50.0

Table 6. Percentage of farmers of different ages that have stopped planting squash, before the study.

Age class of farmers	1-2 years ago (n = 13)	3-6 years ago (n = 13)	7-10 years ago (n = 9)	11-20 years ago (n = 4)
≤ 50 years old	38.5	23.1	33.3	50.0
> 50 years old	61.5	76.9	66.7	50.0

Table 7. Percentage of farmers that had stopped planting squash and those that still cultivate maize.

Age class of farmers	Autlán (n = 30)		El Limón (n = 28)		Ejutla (n = 20)	
	Had stopped	Sown maize	Had stopped	Sown maize	Had stopped	Sown maize
≤ 50 years old	23.3	10.0	17.9	7.1	5.0	5.0
> 50 years old	23.3	13.3	42.9	32.1	35.0	30.0

Table 8. Reasons why farmers have stopped to growing squash (number of individuals).

	Autlán (n = 14)	El Limón (n = 17)	Ejutla (n = 8)
Herbicides use	3	3	4
Low prices of squash	2	4	1
Squash increase work and more cost	1	3	1
Machinery use maize	1	2	1
Milpa shift to other crops	4	3	
Low prices of maize	3	2	1

Table 9. Frequency of the traits (as %) used by farmers to select fruits and seed for sowing.

Trait	CAA as fruit	CMO as fruit	Seeds (both species)
Large size	31	62	69
Form	8	10	0
Weigth	12	37	59
Skin color	11	63	0
Healt	71	78	53
Skin hard texture	0	67	0
Skin soft texture	0	77	0
Pulp thickness	0	67	0
Maturation stage	73	78	0
Pulp dry	0	76	0
Uniformity	0	0	75
Full size	0	0	74
Plump embryos	0	0	78

CAA = *C. argyrosperma* ssp. *argyrosperma*, CMO = *C. moschata*

Table 10. Percentage of farmers that select different number of fruits for planting.

	Autlán (n = 30)	El Limón (n = 28)	Ejutla (n = 20)
Mixed from many fruits	6.6	10.7	20.0
1 to 5 fruits	10.0	17.8	55.0
6 to 10 fruits	33.3	28.6	10.0
11 to 15 fruits	16.7	14.3	5.0
16 to 20 fruits	16.7	14.3	10.0
> 21 fruits	16.7	14.3	0

Table 11. Percentage of farmers using different methods for squash planting.

	Autlán (n = 30)	El Limón (n = 28)	Ejutla (n = 20)
Sown in mixture with maize seed	43.3	64.3	90.0
Sown in mixture in rows	53.3	35.7	10.0
Sown alone in strips	3.3	0.0	0.0

Table 12. Percentage of farmers that have exchange squash seeds for planting

Age class of farmers	Autlán (n = 30)	El Limón (n = 28)	Ejutla (n = 20)
50 or fewer yrs old	20.0	14.3	10.0
Over 50 yrs old	40.0	42.9	60.0

Table 13. Percentage of farmers that have exchanged squash seeds at different intervals of time.

	Autlán (n = 18)	El Limón (n = 16)	Ejutla (n = 14)
Each year	22.2	18.7	14.3
From 2 to 3 years	16.7	12.6	14.3
Occasionally	61.1	68.7	71.4

Capítulo III

Experimental analyses of the opportunity for introgression among three *Cucurbita* taxa in the milpa system

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Abstract

Pollen dispersal was studied by fluorescent dyes as pollen analogues and observations on floral visitation in order to examine the degree of cross pollination among two cultivated squashes, *Cucurbita argyrosperma* ssp. *argyrosperma* and *C. moschata* and their wild relative, *C. argyrosperma* ssp. *sororia*, within a crop field in the state of Jalisco, western Mexico. The wild gourd and domesticated subspecies of *C. argyrosperma* overlapped their blooming period, and both initiated flowering three weeks before *C. moschata*. For the three taxa, an average of 5% ± 1.1 of the open flowers per day were female flowers. The three *Cucurbita* taxa showed similar time in the diurnal flower opening and closing. The most common bee pollinators (*Peponapis azteca, Xenoglossa gabii, Augochlora smaragdina* and *Apis mellifera*) were similar in the three taxa in terms of number of visitors and time of visits. Chemical dyes used as pollen analogues were moved among the three *Cucurbita* taxa. Nearly half of the dyes in each taxon was transferred to the other two taxa, and there were not significant differences among taxa. Dye deposition on stigmas by pollinators did not depend on distance between the anthers acting as source of dyes anthers and the stigmas receiving the dyes.

Key words: cultivated *Cucurbita*, hybridization, introgression, pollination, squash, wild gourd.

Introduction

The opportunity for hybridization between crops and their wild relatives depends firstly on the presence of wild plants viable to cross with the crops under natural conditions. In these cases, if crops and weeds exchange pollen, then hybridization is possible. An intermediate situation exists for those crop species that occur in the vicinity of more distant relatives. For these species, hybridization would depend on the level of cross-compatibility of the species involved (Grant, 1989; Ellstrand, Prentice and Hancock, 1999; Jarvis and Hodgkin, 1999). Because many crops have their evolutionary origins in tropical regions (Smart and Simmonds, 1995), contact with close relatives is much more likely in those regions. Furthermore, although modern agricultural methods such as mechanization are becoming more common in less-developed nations, traditional methods such as slash and burn are still used. These intensive-labor methods usually involve relatively smaller fields (from 1 to 3 has.) surrounded by dense secondary vegetation, thereby increasing contact with wild, weedy relatives (Ellstrand and Hoffman, 1990; Ellstrand, Prentice and Hancock, 1999; Jarvis and Hodgkin, 1999). Transfer of genes from domesticated crops to related weeds, and from wild type to landraces through natural hybridization may have a role in the evolution of squash plants (Jarvis and Hodgkin, 1999).

In Mexico the traditional agricultural system known as "milpa", where corn - beans - squash or corn - squash are cultivated together, generates broad opportunities for gene flow between landraces (traditional and local crop varieties) of each crop species. In milpa system, farmers generally sow more than one landrace in the same or neighboring plots (Lira, 1995; Louette, Charrier and Berthaud, 1997). Moreover, in some regions it is common to find the presence of closely related wild relatives growing near the cultivated plants. Thus it is likely that natural cross-fertilization between wild and cultivated species occurs, as it has been documented in squash (Kirkpatrick and Wilson, 1988; Wilson, 1990; Wilson, Lira and Rodriguez, 1994) and in the other crop component species of the milpa, maize (Wilkes, 1977; Doebley, 1990) and beans (Escalante et al., 1994; Beebe et al., 1997).

In the study zone, the SW of the state of Jalisco, Mexico, the milpa agroecosystem includes maize (*Zea mays* L. ssp. *mays*) and two cultivated taxa of squash, *Cucurbita argyrosperma* Huber ssp. *argyrosperma,* and *C. moschata* (Duch. ex Lam.) Duch. ex Poir., typically planted together within the same plots. Growing nearby it is common by possible to find wild relatives of these crop species, such as teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley), and the wild gourd *C. argyrosperma* ssp. *sororia* (L. H. Bailey) Merrick and Bates. *Cucurbita argyrosperma* and *C. moschata* are very close relatives (Decker et al., 1990; Merrick 1990, 1991; Wilson, Doebley and Duvall, 1992), and there is indirect evidence of high levels of gene flow between them (Montes and

Eguiarte, 2002).

There is experimental evidence of compatibility among these three squash and gourd taxa (Merrick, 1990, 1991, 1997). In both crosses the average number of seeds per fruit was high (Merrick, 1990; 1991; 1997). Interspecific crosses between *C. argyrosperma* and *C. moschata* are capable of producing fertile hybrids, but the seed set was lower than that observed in intraspecific crosses. Both F_1 and F_2 hybrids of the interspecific crosses were highly fertile, with about 89% of viable pollen. The reciprocal cross, when *C. moschata* was used as female parent, did not produce viable embryos in Merrick's (1991) experiments. The cross *C. moschata* x *C. argyrosperma* -with *C. moschata* as female parent- is difficult but not impossible to perform; many pollinations are usually needed for each fruit set, and only few seeds per fruit are typically produced (S. Montes-Hernández, unpublished data; Cutler and Whitaker, 1956; Bemis and Nelson, 1963; Merrick, 1991). Furthermore, the farmers said that it is possible to find hybrids of this combination in their milpas (Merrick and Nabhan, 1984; Zizumbo, 1986; Lira, 1988; Decker-Walters et al., 1990; Lira, 1995; Merrick, 1995).

This study was motivated by a concern on the possibility of pollen flow between landraces of cultivated squash, and their wild relatives in order to understand the participation of the wild type in the genetic diversity of cultivated squashes. The present study addressed the following questions: Are there conditions for pollen flow among cultivated squash species and between wild gourd and cultivated squash within the milpa agroecosystem? How do the reproductive ecology and phenology of the squash plants influence the levels of gene flow between the cultivated and wild types? Is the pollen flow among these squash taxa mediated by pollinator activity?. We test the hypothesis that opportunity for introgression among three *Cucurbita* will not differ because the patterns of pollinator movements are similar in three taxa. If introgression occurs, we expect that pollen flow between transgenic cultivated squash and their wild relatives is possible (Wilson, 1990; Spencer and Snow, 2001). However, before this risk can be assessed, studies that examine the extent of introgression between the wild and cultivated plants are necessary (Payne, 1997), given that in this genus introgression is very common (Wilson, 1990); as appears to be the case in maize (Quist and Chapela, 2001).

Materials and methods

The studied taxa

Squashes (*Cucurbita* spp.) are among the most important traditionally cultivated plants from South Mexican and Central America center (Whitaker, Cutler and MacNeish, 1957; Whitaker and Davis, 1962; Harlan, 1975). Squashes are monoecious plants, with both staminate and pistillate flowers in different part of the vine, and requiere pollinators to set fruit, as they are never apomictic. Flowers of both sexes last only one morning. Pollination and floral biology of squashes have been reviewed by Whitaker and Davis (1962) and Robinson and Decker-Walters (1997), who found that pollination is carried out by insects, which in the Americas are mainly specialist bees of the genera *Peponapis* Robertson and *Xenoglossa* Smith (Hurd and Linsley, 1964; 1966; 1967; Hurd, Linsley and Whitaker, 1971).

Two cultivated squash species were included in this study: *C. argyrosperma* ssp. *argyrosperma* (CAA), and *C. moschata* (CMO), which are widely cultivated in the study area as part of the milpa agroecosystem. The weedy and wild type *C. argyrosperma* ssp. *sororia* (CAS) grows nearby cultivated plots and even within them (Merrick, 1991, 1997; Merrick et al., 1996; Bautista, 1997).

Study site

A study site was established in a maize plot at the village of El Chante ($19^0$43'09" N, $104^0$12'24" W), at elevation 991 m., in the municipality of Autlán, in the state of Jalisco, Mexico. The owner of this plot had planted both squash species (*C. argyrosperma* ssp. *argyrosperma* and *C. moschata*) in association with maize in the rainfall season (June-November). Some plants of wild gourd occurred in and around the milpa plot. Fieldwork was carried out from September to October 1999, when the squash plants were flowering. The conditions of the study site were the same normally faced by farmers, since we wanted to analyze the opportunity for gene flow among *Cucurbita* species in this type of setting.

Floral and insect behavior

Flower anthesis and pollinators activity were observed directly from early morning (6:00 a.m.) to noon (12:00 a.m.). Pollinator behavior was studied recording insect visitors in one staminate and one pistillate flower, during three days (3-5 October, 1999) for each *Cucurbita* taxon. Individual staminate and pistillate flowers of all three *Cucurbita* taxa were marked previously (before day) for all observations, in order to recognize them easily the day after early morning. Insect visits in marked flowers in the three *Cucurbita* taxa were recorded during the same time period per day, and individual insects on open flowers were collected and identified. The number of male vs. female flowers in anthesis per day during the period of maximum flowering was counted in four different 100 m^2 quadrats, one per day, during four days (27-30 September 1999) within the milpa field.

Estimation of dye movement

Fluorescent dyes as pollen analogues were used to investigate the range and directionality of pollen flow (orange G, methylene blue and light green, Kearns and Inouye, 1993, pp. 136-140; Eguiarte et al., 1993; García-Franco et al., 1998) on different numbers of staminate flowers in each *Cucurbita* taxon during a set of five days (24 and 26-29 1999, Table 4). The analogues were supplied in the morning at the starting point of anthesis. A specific color of pollen analogue was assigned to each *Cucurbita* taxon, and these taxon-specific colored dyes were used throughout the course of the study, in order to sampling the dye movement for each taxon. Location of each female flower in anthesis in the milpa was marked the same day with flags of different colors, to identify them the following afternoon. After 24 hr we collected all previously marked stigmas from the study site and checked under a longwave ultraviolet light whether they had any colour dye or not.

We mapped the position of all the open pistillate flowers in the milpa plot, recording those stigmas that were dyed and those that were not dyed. Distances traveled by dye were averaged for each *Cucurbita* taxon per day. Because number of staminate flowers on which we put fluorescent dyes varied in each taxon, and there were different numbers of stigmas available per taxon per day, we calculated the number of stigmas of each taxon expected to possess dye from different sources (taxon). We performed this calculation for each pair of combinations (nine combinations in total) in the following way: (number of anthers with a taxon-specific dye / total number of anthers with all colors of dye) x (stigmas that were marked with one particular taxon-specific dye / total number of stigmas sampled) x (total number of stigmas with all colors of dye).

To investigate the distance traveled by pollen, we applied fluorescent dyes in all the staminate flowers of the wild type (*C. argyrosperma* spp. *sororia*) in an area of 30 x 5 m close to the road in El Chante where the wild gourd was abundant. We collected all the flowers in each 30 x 5 m interval along the road, up to 180 m in both directions from area with dyed flowers (north and south), and checked with UV light whether the stigmas were dyed or not.

Results

Floral and insect behavior

Both subspecies of *C. argyrosperma* showed similar date of blooming start, which started three weeks before *C. moschata*. *Cucurbita moschata* finished its flowering period two months later than the two subspecies of *C. argyrosperma*. The total proportion of pistillate flowers differed among the three *Cucurbita* taxa during the five days in which this characteristic was assessed (see below), but on average pistillate flowers of *C. moschata* were the most abundant (47.4%), followed by the wild *C. argyrosperma* spp. *sororia* (29.3%) and *C. argyrosperma* spp. *argyrosperma* (23.3%).

We found an average (in four days) of 43.1 ± 18.3 open staminate flowers per day, in a 100 m^2 plot, but only 2.2 ± 1.2 pistillate open flowers (5.0%). The three *Cucurbita* taxa showed similar time in the flower opening and closing, with an average 6:30 am and 11:40 am, respectively.

The insect pollinators that were most abundant during the year of the experiment were *Peponapis azteca* Hurd and Linsley (70.23%), *Xenoglossa gabii* Cresson (10.96%) [Table 1]. Flowers of *C. argyrosperma* spp. *sororia* were targeted by most of the visits of *Peponapis azteca*. For *Xenoglossa gabii* and *X. fulva* we found significant differences in frequency of visits among *Cucurbita* taxa. *Cucurbita moschata* was more visited by these bee species than expected by chance, but the difference may be an artifact due to the low number of visits, in particular by *X. fulva* (Table 1). *Peponapis azteca*, *X. gabii* and *X. fulva* are considered as specialists in interaction with flowers of *Cucurbita* species (Hurd, Linsley and Whitaker, 1971) and were found in both male and female flowers (Table 2). Staminate flowers received more visits than the pistillate flowers (442 and 297 visits, respectively), but this difference was not significant among pollinators and *Cucurbita* taxa

(Table 2). The three mentioned bees begin their visits to the squash flowers generally earlier than others bees (Table 3).

Dye movement

Dyes were carried by bees from marked anthers to stigmas of female flowers, for at least 180 m, the maximum distance (the most remote point evaluated in this study) in which we found stigmas dyed (Fig. 1).

During five days the number of staminate flowers used as sources for the fluorescent dyes and the number of stigmas receiving those taxon-specific dyes was variable (Table 4). The percentage of dyed stigmas did not vary significantly among the three *Cucurbita* taxa ($F = 0.401$; $p = 0.687$) [Table 4].

The quantity of dyed stigmas for each taxon depended either on pollen source or pollinator preference to particular plant taxa, because all combinations were significantly different (Table 5). The number of stigmas showing dye from each taxon, in five days, were significantly different among taxa (Table 5). When evaluated across all taxa and all pollen sources, the number of dyed stigmas was found to be significantly different (Table 5). Only between 27.6% and 50.0% of dyed stigmas were colored by the same taxon source, and *C. moschata* showed the highest value. The mean percentage of movement of pollen from each taxon to the three taxa was significantly different among *Cucurbita* taxa (Table 5).

Across *C. argyrosperma* spp. *argyrosperma* and *C. moschata*, dye deposition on stigmas by pollinators did not depend on the distance between the source anthers and the stigmas receiving the dyes (Table 6). *Cucurbita argyrosperma* spp. *sororia* did not fit this general trend as the distances were very short in movement from staminate to pistillate flowers (average 1.48 m, ± 0.49, range from 0.8 m to 2.5 m), because the *C. argyrosperma* spp. *sororia* plants were in a small area close the edge in the study area of the milpa field. When this last taxon was used as dye source and dye receiving, differences were observed in the mean distance among the three taxa ($F = 25.81$, $P < 0.0001$; $F = 44.89$, $P < 0.000$, respectively) [Table 6]. When *C. argyrosperma* spp. *argyrosperma* and *C. moschata* were used as pollen source, no significant differences were observed in the mean distance to which pollinators moved dyes from source anthers to stigmas of the three taxa ($F = 1.62$, $p = 0.217$ and $F = 1.05$, $p = 0.366$; respectively) [Table 6].

Discussion

Floral and insect behavior

Bees were abundant in both staminate and pistillate flowers of the three taxa, but - with the exception of *Xenoglossa gabii* and *X. fulva* - which apparently exhibited a preference for *C. moschata*, bees species not have clear preference for a particular *Cucurbita* taxon. On the other hand, *Peponapis azteca* showed a slight preference for *C. argyrosperma* spp. *sororia*. However, all bees visited the flowers of the three taxa, giving opportunity to pollen movement among the *Cucurbita* plants. Several studies have focused on the participation of bees in pollination in *Cucurbita*, and in particular by the *Cucurbita* -specialist species of the genera *Peponapis* and *Xenoglossa* (Hurd, Linsley, and Whitaker, 1971; Ordway et al., 1987; Merrick, et al., 1996; Merrick, 1997; Canto-Aguilar and Parra-Tabla, 2000). Nevertheless, under the conditions of our study, bees did not have clear preference for a given taxon of *Cucurbita*. Besides the specialist pollinators of *Cucurbita*, also the honey bee (*Apis mellifera*) was recorder in most of the flowers, a finding that coincides with results reported by Avila *et al.* (1989), Tepedino (1981), Merrick et al. (1996), Merrick (1997) and Bautista (1997).

The larger number of *C. moschata* plants in the field compared to that of *C. argyrosperma* was reflected in the high number of staminate and pistillate flowers of the first taxon. The larger number of staminate than pistillate flowers in the three taxa could allow each pollinator to visit great number of anthers before they visited the stigmas. This differences between staminate and pistillate flowers numbers have been reported in *C. pepo* (Nepi and Pacini, 1993) and in other Cucurbitaceae (*Apodanthera undulata* Gray) (Delesalle and Buchmann, 1991). In addition, in *A. undulata* every day there was synchronization in the starting of flowering in both staminate and pistillate flowers. Greater pollen loads could enhance the potential for fertilization, and the pistillate flower has the option of being able to select the pollen that is more compatible or that develops better. This has been documented in other *Cucurbita* species, where the load of pollen received by the stigma influences the vigor of the offspring (Quesada, Winsor and Stephenson, 1996; Schlichting et al., 1990). A higher proportion of selfed progeny has been observed in mixed population of the cultivated zucchini type *C. pepo* and the wild gourd *C. pepo* ssp. *ovifera* var. *texana* (Wilson and

Payne, 1994); the stigmas chose the genotype which grows the pollen tube faster and that are more compatibility among them. The possibility to select pollen by the stigmas may favor intraspecific crosses among the three *Cucurbita* species present in the field. On the other hand, the fact that there is no difference in the time of the day in which anthesis occurs among taxa favors the movement of pollen among them, as discussed by Bautista (1997), Merrick et al. (1996), Merrick (1997) for our *Cucurbita* taxa, and by Wilson, Lira and Rodriguez (1994) for *C. argyrosperma* spp. *argyrosperma* (cultivated) and *C. fraterna* (wild gourd). In terms of frequency of visits to staminate flowers, our results contrast with those reported by Merrick (1997). Also, Canto-Aguilar and Parra-Tabla (2000) found that *C. moschata* staminate flowers received more visits of *Peponapis limitaris* than the pistillate ones.

Dye movement

The use of fluorescent powders provides an indirect indication of actual pollen dispersal, and there are reports that fluorescent powders in general terms function well as surrogate pollen in *Cucurbita* (Ordway et al., 1987) and other plants (Kearns and Inouye, 1993; Eguiarte et al., 1993; García-Franco et al., 1998).

Pollen can be moved for at least 180 m under the conditions of study. Also, on average, half of the taxon-specific pollen analogues were moved to stigmas of other taxa, indicating that pollen flow within and among three *Cucurbita* taxa in a single location may be rampant, as suggested in populations genetics studies by Montes and Eguiarte (2002).

The relative abundance of pistillate flowers of *C. moschata* in anthesis per day increased the possibility of the bee visitations to this species. When we calculated the percentage of stigmas dyed in each taxon, there were significant differences among taxa. *Cucurbita moschata* exhibited the highest number of dyed stigmas. Therefore, *C. moschata* appeared to have received more bee visits. Nevertheless, the identity of *C. moschata* can be maintained because the possibility of fertile interspecific hybrids resulting from cross pollination when *C. moschata* is the female flower is limited (Merrick, 1991). In contrast, it is relatively easy to produce fertile hybrids between the two subspecies of *C. argyrosperma* (Merrick, 1990; 1991). On the other hand, there was a slight preference of pollinators to travel from staminate flowers of *C. argyrosperma* ssp. *sororia* to pistillate flowers of the other taxa.

Pollinator flight patterns are influenced by flower density and distribution and by the richness of the floral rewards of both pollen and / or nectar (Levin, Kerster and Niedzlek, 1971; Schmitt, 1983; Harder and Barrett, 1996). Thus, less pollen movement might be expected in large and dense fields with many flowers where bees tend to forage on the next nearest flower (nearest neighbor) and remain in a limited foraging area, than in smaller fields where flowers are sparse or distantly spaced (Levin, Kerster and Niedzlek, 1971; Schmitt, 1983; Ordway et al., 1987; Harder and Barrett, 1996).

The pollinator visits and the movement of fluorescent dyes among the three taxa, and the range of pollen flow reveal that the pollinators' movements may be a key factor in determining the potential for gene flow among *Cucurbita* taxa in the milpa. We considered that this high level of gene flow contributes to the low genetic differentiation among *Cucurbita* taxa within a given population in the region (Montes-Hernández and Eguiarte, 2002). But this study also indicates that there may be large opportunities for hybridization between transgenic cultivated squash and their wild relatives (Wilson, 1990; Spencer and Snow, 2001). Therefore, we would recomend that, due to the large diversity of *Cucurbita* in Mexico, and given the suggestion of Quist and Chapela (2001) of extensive gene flow among transgenic and maize landraces in Southern Mexico, where a high diversity of maize is known to exist, we must be extremely cautious in the use of transgenic squash plants in Mexico, where the center of origin and diversification of this genus is found, which could contaminate wild relatives, landraces of the same species, and even others related species.

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Table 1. Total number of bees that visited staminate and pistillate flowers in three taxa of *Cucubita* during three observation days in each taxon at El Chante, Jalisco state, Mexico. Results of the G-Test for Goodness of fit (G), with correction of continuity (Sokal and Rohlf, 1995). Expected frequencies are shown in parentheses. CAA = *C. argyrosperma* ssp. *argyrosperma*, CMO = *C. moschata*, and CAS = *C. argyrosperma* ssp. *sororia*. This taxon abbreviations are used throughout the text.

Pollinator type	CAA	CMO	CAS	G	*P*
Peponapis azteca	177 (171.4)	133 (155.9)	209 (191.7)	5.24	0.073
Xenoglossa gabii	17 (26.7)	37 (24.3)	27 (29.9)	10.08	0.006
X. fulva	5 (4.9)	9 (4.5)	1 (5.5)	9.12	0.010
Apis mellifera	11 (10.9)	13 (9.9)	9 (12.2)	1.79	0.409
Augochlora smaragdina	14 (11.6)	11 (10.5)	10 (12.9)	1.23	0.542
Others	20 (18.5)	19 (16.8)	17 (20.7)	1.09	0.579

Table 2. Total number of bees visitors to both staminate and pistillate flowers; and results of the G-Test for Goodness of fit (G), with correction of continuity (Sokal and Rohlf, 1995) for pollinators by flower sex in each three taxa of *Cucurbita*, during three days. NS = Not significant difference.

	CAA		CMO		CAS	
Pollinator type	♀	♂	♀	♂	♀	♂
Peponapis azteca	81	96	47	86	80	129
Xenoglossa gabii	7	10	13	24	11	16
X. fulva	2	3	3	6	1	0
Apis mellifera	4	7	6	7	3	6
Augochlora smaragdina	6	8	4	7	6	4
Others	11	9	7	12	5	12
G	1.336 NS		0.635 NS		4.559 NS	

Table 3. Time of starting and finishing of activity of different pollinators in three taxa of *Cucurbita*, in both staminate and pistillate flowers (n = 3 flowers per sex flower), during three days.

	Cucurbita taxa					
Flower sex / Pollinator	CAA		CMO		CAS	
Staminate	Start	End	Start	End	Start	End
Peponapis azteca	7:38	10:45	7:29	10:32	7:24	10:12
Xenoglossa gabii	6:51	8:26	7:02	9:52	7:16	10:17
X. fulva	6:58	8:30	7:09	7:49	---	---
Apis mellifera	8:23	8:52	8:49	9:12	8:12	9:20
Augochlora smaragdina	8:13	10:42	8:33	11:15	8:46	11:13
Pistillate						
Peponapis azteca	7:12	10:22	7:45	10:31	7:19	10:05
Xenoglossa gabii	7:01	8:31	7:12	9:34	7:04	8:21
X. fulva	6:37	7:49	7:24	9:50	7:10	7:12
Apis mellifera	8:12	10:01	8:10	10:52	8:08	10:17
Augochlora smaragdina	8:57	11:03	9:01	11:02	9:36	11:20

Table 4. Number of staminate flowers on which dyes were applied (♂D), total number of examined pistillate open flowers in the plot (♀) and percentages of total pistillate flowers with dye (♀D) from all sources, for each taxon during five days.

	CAA			CMO			CAS		
	♂D	♀	%♀D	♂D	♀	%♀D	♂D	♀	%♀D
Total	31	46	41.30	83	94	45.74	70	58	58.62
Mean	6.2	9.2	40.76	16.6	19.0	45.58	14.0	11.6	47.92
SD	3.03	0.84	18.35	14.38	4.85	12.71	11.94	5.68	30.23

Table 5. Number (and percentages) of pistillate flowers with dye from different taxa after five days of labeling; and results of the G analysis for Goodness of fit (G), by correction of continuity (Sokal and Rohlf, 1995).

	Pollen source				
Stigmas dyed	CAA	CMO	CAS	G	P[b]
CAA	8 (27.6)	4 (15.4)	7 (17.2)	11.32	0.023
CMO	13 (44.8)	13 (50.0)	17 (41.4)	17.78	0.0014
CAS	8 (27.6)	9 (34.6)	17 (41.4)	23.78	0.00009
G	37.14	11.56	27.14		
P[a]	< 0.00001	0.021	0.00002		

[a] Significance levels of the G test in differences between pistillate flowers with dye, when each *Cucurbita* taxon was used as a source pollen by *Cucurbita* taxa.

[b] Significance levels of the G test in differences between pistillate flowers of each *Cucurbita* taxon, dusted with dye from different pollen sources.

Table 6. Average distance in meters (± standar desviation), and results of the analysis of variance (ANOVA) between the anthers dyed (pollen source), and stigmas with dye, per taxon of *Cucurbita* during five days.

	Pollen source				
Stigmas dyed	CAA	CMO	CAS	F	P[b]
CAA	8.7 (± 5.55)	13.02 (± 9.81)	22.61 (± 13.99)	3.46	0.055
CMO	10.61 (± 7.33)	11.22 (± 8.81)	15.94 (± 7.70)	2.09	0.135
CAS	5.94 (± 1.49)	7.35 (± 2.75)	1.48 (± 0.49)	44.89	< 0.00001
F	1.62	1.05	25.81		
P[a]	0.217	0.366	< 0.00001		

[a] Significance levels of the ANOVA in differences between pistillate flowers with dye, when each *Cucurbita* taxon was used as a source pollen by *Cucurbita* taxa.

[b] Significance levels of the ANOVA in differences between pistillate flowers of each

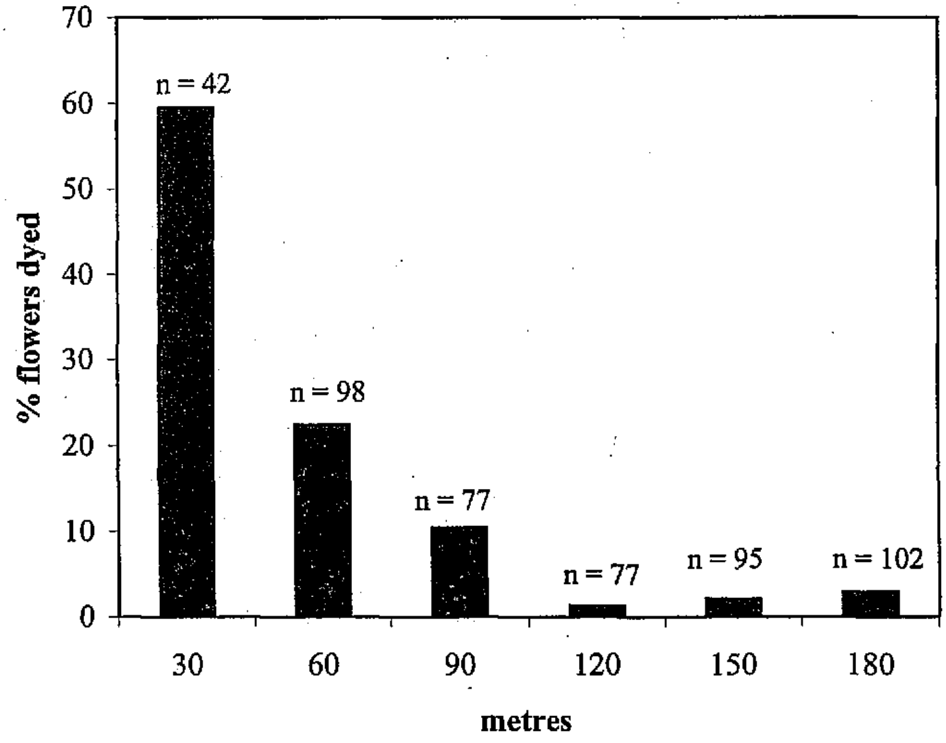
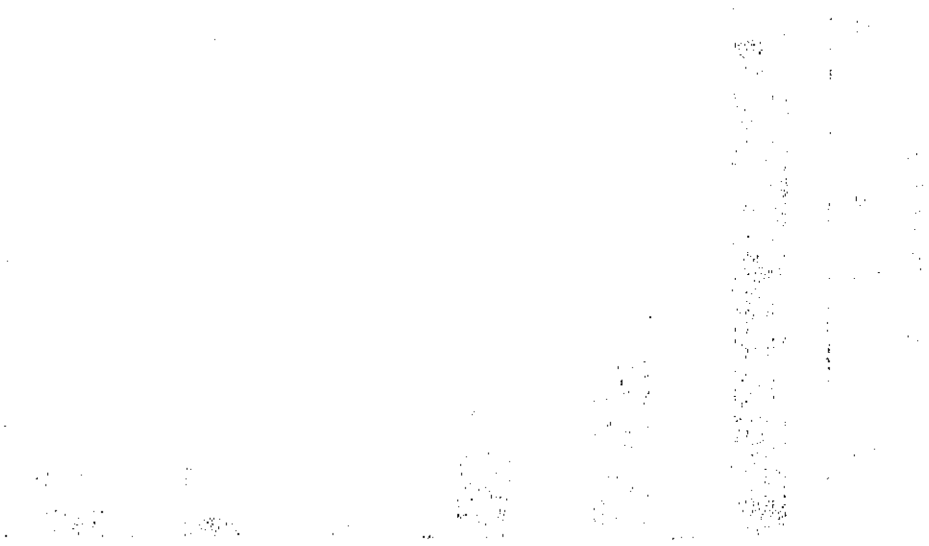


Fig. 1. Percentage of number of staminate and pistillate flowers with dye, from *Cucurbita argyrosperma* ssp. *sororia* wild gourd plants collected at 30 x 5 m intervals in northward and southward directions along the road in El Chante, Jalisco, México. n = total number of both staminate and pistillate flowers found in each interval area.



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Capítulo IV

Este capítulo se encuentra como artículo en prensa, en la revista American Journal of Botany 89(7): 2002.

GENETIC STRUCTURE AND INDIRECT ESTIMATES OF GENE FLOW IN THREE TAXA OF *CUCURBITA* (CUCURBITACEAE) IN WESTERN MEXICO[1]

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Cultivated squash (*Cucurbita argyrosperma* ssp. *argyrosperma* and *C. moschata*) are important in the Mexican traditional agroecosystem. They are typically cultivated within maize fields where adjacent populations of a wild, close relative, *C. argyrosperma* ssp. *sororia*, occur. Consequently, there are ample opportunities for gene flow between domesticated and free-living *Cucurbita* populations. We used allozymes to examine genetic variation and gene flow among these three *Cucurbita* taxa in the state of Jalisco in Western Mexico. Twelve polymorphic allozyme loci were used to calculate genetic diversity for 16 populations of *Cucurbita*. We found high levels of genetic variation: polymorphism of 0.96, mean allelic diversity of 2.08 and average expected heterozygosity 0.407, and little differentiation among conspecific populations ($D = 0.081$; $F_{ST} = 0.087$; $N_em = 5.22$). These findings indicate that *Cucurbita* possess a high pollen dispersal potential, a somewhat surprising result considering they have specialist pollinators. UPGMA analysis of allozymes suggests the existence of at least two distinct groups of populations, one consisting of both subspecies of *C. argyrosperma* and another consisting of *C. moschata*.

Key words: *Cucurbita*; Cucurbitaceae; cultivated squash; gene flow; genetic diversity; genetic structure; wild gourd.

The ecological and cultural conditions under which traditional agriculture is carried out in Mexico have helped to preserve large amounts of diversity in locally cultivated species (Bye, 1993; Hernández, 1993; Souza et al., 1997). This variation is complemented by the presence of their wild relatives within the agroecosystems (Harlan, 1976). These wild relatives have many important influences on their cultivated relatives, and store great amounts of genetic variation (Doebley, 1992; Escalante et al., 1994; Colunga et al., 1999), which may be of interest for future crop improvement programs (Doebley, 1990; Wilson, 1990; Jarvis and Hodgkin, 1999). In addition, they can provide information on the domestication process (Doebley, 1990), and they represent a potential source of gene flow with their genetically engineered relatives (Doebley, 1990; Ellstrand and Hoffman, 1990; Wilson, 1990; Hancock, Grumet, and Hokanson, 1996; Hokanson, Hancock, and Grumet, 1997; Ellstrand, Prentice, and Hancock, 1999).

In Mexico, squash (*Cucurbita* spp.) is a very important traditional crop and large amounts of genetic variability have been maintained. *Cucurbita* spp. are of great nutritional and economic importance for Mexicans; they are used both as an immature fruit (zucchini type) and as a mature fruit and as seeds (Whitaker and Bohn, 1950; Lira, 1995; Merrick, 1995).

The cultivated species are monoecious, and they are pollinated by various wild specialist bees, mainly *Peponapis* spp. and *Xenoglossa* spp. (Hurd, Linsley and Whitaker, 1971). In the traditional mesoamerican agricultural systems, called "milpa", maize - beans - squash or maize - squash are cultivated together. In these milpa fields, it is common to find wild relatives growing near the cultivated plants. Thus, it is likely that natural cross-fertilization between wild and cultivated species occurs, and indeed, such gene flow has been documented in maize (Wilkes, 1977; Doebley, 1990), beans (Escalante et al., 1994; Beebe et al., 1997), and squash (Kirkpatrick and Wilson, 1988; Wilson, 1990; Wilson, Lira and Rodriguez, 1994).

In the southwestern part of the state of Jalisco, Mexico, the milpa agroecosystem includes cultivated maize (*Zea mays* L. ssp. *mays*) and two cultivated taxa of squash, *C. argyrosperma* Huber ssp. *argyrosperma* and *C. moschata* (Duch. ex Lam.) Duch. ex Poir., growing near their wild relatives, *Z. mays* ssp. *parviglumis* Iltis & Doebley or teosinte, and the wild gourd *Cucurbita argyrosperma* ssp. *sororia* (L.H. Bailey) Merrick & Bates.

Experimental evidence has revealed that the taxa involved in the present study can be crossed to produce fertile seeds (Merrick, 1990, 1991; Wessel-Beaver, 2000). A close relationship exists among *C. argyrosperma* ssp. *argyrosperma* (mean diversity [D] = 0.02 [0.00-0.06]) and *C. argyrosperma* ssp. *sororia* populations (D = 0.01 [0.00-0.06]; Decker, 1986), although *C. moschata* populations are more distant (D = 0.24 [0.16-0.32]; Wilson, 1989; Wilson, Doebley and Duvall, 1992). On the other hand, data on the genetic diversity in extant populations indicate a close relationship between *C. argyrosperma* ssp. *argyrosperma* and *C. argyrosperma* ssp. *sororia* (average D = 0.03) and greater differentiation between *C. argyrosperma* ssp. *argyrosperma* and *C. moschata* (average D = 0.22; Wilson, 1989; Merrick, 1991). A study of genetic diversity in *Cucurbita* revealed that *C. moschata* has greater genetic diversity (mean expected heterozygosity, H = 0.052) than *C. argyrosperma* (0.039), although the sample size was small in both species [8 - 20 individuals; with 14 loci] (Decker-Walters et al., 1990). Decker (1986) and Decker-Walters et al. (1990) present evidence that the two species have introgressed. In the common zucchini, the *C. pepo* L. complex, genetic diversity and heterozygosity are moderately high (D = 0.17 and H = 0.089; Decker and Wilson, 1987) and alleles from the cultivated species have been found in wild populations. This has been interpreted as evidence of gene flow among wild and cultivated populations (Decker and Wilson, 1987; Kirkpatrick and Wilson, 1988; Wilson, 1990).

Our main objective was to determine the levels of genetic variation within and among taxa of *Cucurbita* in Jalisco, Mexico, and to analyze their relationships. Specifically, we assessed (1) the levels of gene flow within and among populations of two different species of squash (both of which are cultivated in the traditional milpa agroecosystem) and a wild type that grows in adjacent fields, (2) the distribution of allozyme variation within and among populations of the wild and cultivated squashes, and (3) the genetic relationships among populations of the three taxa.

MATERIALS AND METHODS

Study sites and plant material-- For the allozyme analyses, seeds were collected from plots of cultivated squash in the southwestern part of the Jalisco State in Mexico. In these sites *C. argyrosperma* ssp. *argyrosperma* and *C. moschata* are sown together, but the weedy *C. argyrosperma* ssp. *sororia* is also present. For electrophoretic survey, we used seeds from all three taxa, and a population of *C. pepo* as an outgroup. We chose six localities in five municipalities (Fig. 1). The first three taxa were sampled within four of the localities (San Miguel, San Lorenzo, El Chante y Los Parajitos; Table 1). The CAA8 population of *C. argyrosperma* ssp. *argyrosperma* and the *C. pepo* population were collected in Tepec, outside of the study region in an area where weedy *Cucurbita* species are not found (Table 1). In all, we sampled a total of 16 populations including 6 of *C. argyrosperma* ssp. *argyrosperma*, 4 of *C. moschata*, 5 of the wild type *C. argyrosperma* ssp. *sororia*, and 1 of *C. pepo* (Table 1). Landraces (traditional and local crop varieties) were sampled from seeds donated from several local farmers. An average of 4 - 5 fruits per population of wild type squash were collected haphazardly near the milpas; all of the seeds from each population were combined and 45-50 seeds from each batch were randomly sampled and sown in organic growing medium (1:1, humus:fertile soil). These seedlings were left to grow in a greenhouse and young leaf material harvested for electrophoresis (see below). On average, protein from 45 seedlings per population, 720 in total, were subjected to electrophoretic analyses.

Isozyme electrophoresis-- Starch gel horizontal electrophoresis was used to estimate genetic variation in allozyme loci within and among selected populations of *Cucurbita*. Young leaf material of all sampled individuals was ground with an electric drill, using 3-4 drops of an extraction buffer, composed of a 3:1 mixture of the Veg II buffer of Pitel and Cheliak (1984) and the extraction buffer of Yeh and O'Malley (1980). The extract was absorbed on 15 x 2 mm Whatman 1MM filter paper wicks and stored in an ultracold freezer (-80°C) until electrophoresis.

Several tray-gel buffer systems were tested to determine which provided the best resolution for each enzyme locus, and several loci of many enzymes were screened to determine which were polymorphic. Alleles and loci were assessed following the techniques outlined in earlier studies of *Cucurbita* spp. (Decker, 1986; Decker and Wilson, 1987; Kirkpatrick and Wilson, 1988; Decker-Walters et al., 1990, among others). Putative loci were assigned sequentially, with the most anodally migrating isozyme designated as 1, the next 2, etc. Likewise, alleles were assigned sequentially with the most anodally migrating allele designated as 1, the next 2, etc. Two gel and electrode buffer systems were used to assay the following allozymes: phosphoglucose isomerase (Enzyme commission number (E.C.) 5.3.1.9, PGI, two loci), leucine aminopeptidase (E.C. 3.4.11.1, LAP, one locus), esterase (E.C. 3.1.1, EST, one locus), and peroxidase anodical (E.C. 1.1.1.7, APX, three loci; cathodal and anodal), in 11 % starch gels (375 mL) ran in LiOH buffer pH 8 (Soltis et al., 1983), at 60 mA for 6-7 h. We analyzed isocitrate dehydrogenase (E.C. 1.1.1.42, IDH two loci), malate dehydrogenase (E.C. 1.1.1.37, MDH one locus), phosphoglucomutase (E.C. 2.7.5.1, PGM, one locus), and malic enzyme (E.C. 1.1.1.40, ME, one locus), on the same gels run with a histidine-citrate buffer pH 6.5 system (Cardy, Stuber and Goodman, 1980), at 30 mA for 7-8 h. Allozyme markers for 12 enzyme loci for the four taxa were consistent and interpretable.

Data analysis-- Allelic frequencies in each population were calculated from isozyme phenotypes. Allelic variation within and across all populations was quantified by calculating the following statistics for each locus in each population: percentage of polymorphic loci ($\hat{p}$), polymorphic index (PI; proportion of polymorphic loci per population), mean number of effective alleles per locus (A), mean observed heterozygosity (H_o; direct estimate), Hardy-Weinberg expected heterozygosity (H_e), and the fixation index (F; Wright, 1965). All estimates were calculated with BIOSYS (Swofford and Selander, 1981).

The distribution of genetic variation within and among populations was estimated by calculating Wright's (1951) F statistics (F_{ST}, F_{IS}, and F_{IT}) from variance components using Weir and Cockerham's (1984) estimators θ, f, and F. These tests assume that the populations are in mutation-drift balance and share the same time of divergence from a common ancestor. Here we derive F-statistics both within taxa and among all populations of the three taxa. For the second analyses, we must therefore assume that the three taxa share a recent common ancestor and that the time of divergence of each taxa from this ancestor is approximately the same. Given the inter-crossability of the taxa and the high similarity of their isozyme profiles, this is probably a reasonable assumption. The deviation of fixation index (F) from zero for each locus in each population (in total 192 tests; 12

loci per 16 populations) was tested using a chi-square test (Li and Horvitz, 1953), and 95% CI intervals were estimated by 1000 bootstraps with the program TFPGA (Tools for Population Genetic Analyses; Miller, 1997). For each population in each taxon, the number of loci that exhibited an excess or deficit of heterozygotes based on Hardy-Weinberg (H-W) expectation was noted.

The rate of interpopulation gene flow within taxa and among all populations was estimated using the procedure based on Wright's F_{ST}, where $F_{ST} = (1/4N_em + 1)$ (Wright, 1951). This method estimates N_em, which is the average number of migrants into a population per generation. In this case, we used our estimate of θ to estimate N_em following Crow and Aoki (1984), $N_em = [(1/\theta)-1]/4\alpha$, where $\alpha = (n/n\text{-}1)^2$, and n = number of subpopulations. The estimate of N_em assumes that there are equal quantities of migration between all populations. Although this assumption is probably not satisfied, the fact that we have approximately equal numbers of populations per taxa and representatives of each taxa in each locality, making this assumption is also reasonable.

The genetic divergence between populations was determined using Nei's genetic distances (D) [Nei, 1978], which is an index of genetic similarity. Dendograms for all taxa together were constructed using UPGMA (unweighted pair group method using arithmetic means) and the confidence of each node was tested with bootstraps. The majority of the analyses were carried out using the computer program TFPGA (Miller, 1997), unless indicated otherwise.

RESULTS

Genetic diversity-- We found that the eight enzymes coded for 12 loci (Table 2), all of which were polymorphic. The allele frequencies at each locus in each of the 16 populations of the four *Cucurbita* taxa studied are given in Table 2. The average effective number of alleles per locus is 2.08 (Table 3). Twenty-two of 31 alleles occurred in all *Cucurbita* populations (Table 2). Four of the 16 populations harbored a private allele, each one in a distinct taxon, including one in the outgroup taxon, *C. pepo*. In *C. argyrosperma* ssp. *argyrosperma* there were five rare alleles, and one population, CAA11, had two rare alleles and a single private allele (Table 2). Mean percentage of polymorphic loci, $\hat{p}$, was slightly higher in *C. argyrosperma* ssp. *sororia* (1.0) than in *C. moschata* (0.97) or in *C. argyrosperma* ssp. *argyrosperma* (0.93; Table 3). On average, 96% of the loci were polymorphic in each population and the mean expected heterozygosity was 0.407. The mean expected heterozygosity was higher again in *C. argyrosperma* ssp. *sororia* (H_e = 0.426) and lower in *C. argyrosperma* ssp. *argyrosperma* (H_e = 0.391), while the average expected heterozygosity in *C. moschata* was intermediate, 0.416 (Table 3).

Of the 192 X^2 tests of the deviation of F from the expected value per locus per population, 94 loci-population combinations had an F value significantly different from zero. Of these 94 tests, 39 were positively different from zero, and 55 were negatively different from zero ($\alpha \leq 0.05$ to < 0.005; Tables 3, 4). Sixty percent of the populations of *C. argyrosperma* ssp. *sororia*, 75% of *C. moschata*, and 50% of *C. argyrosperma* ssp. *argyrosperma* had average within-population F values that were significantly negative, indicating an excess of heterozygotes (Table 3). In both subspecies of *C. argyrosperma*, the mean F values averaged across all populations were positive or nearly zero (0.006 in *C. argyrosperma* ssp. *sororia*; -0.046 in *C. argyrosperma* ssp. *argyrosperma*), while in *C. moschata* the average F values were negative (-0.174). The number of loci in each *Cucurbita* population with F values significantly different from zero ranged from three in population CAA7, to 11 in population CAA6 (Table 3). The average number of loci that had F values significantly different from zero was similar in the three taxa (*C. argyrosperma* ssp. *sororia* = 6.4, *C. argyrosperma* ssp. *argyrosperma* = 5.5, and *C. moschata* = 6.0; Table 3), although when the F values were calculated over all 12 loci, only one population (CMO12) showed a significant departure from H-W equilibrium (95% CI; data not shown). The loci that exhibit the greatest departure from H-W equilibrium were *Apx*2 (11 populations) and *Pgi*2 (16 populations; Table 4).

Similarly, analyses of Wright's F statistics for each locus showed highly significant departures from zero for many loci (Table 4). However, values of F_{IS} and F_{ST} calculated by taxon or locality are low, implying that there are small deficiencies in homozygotes globally and among populations. F_{IS} and F_{IT} values were significantly different from zero for nine and eight loci, respectively; four loci were negatively significant for F_{IS} and three for F_{IT}, averaged over all *Cucurbita* populations (Table 4). Eleven of the 12 loci had significant F_{ST} values (Table 4). Table 5 presents F statistics evaluated by taxon (CAS, CAA or CMO) and then by locality (San Miguel, San Lorenzo, El Chante y Los Parajitos). The analyses by locality included one population of each taxon resident in that locality. All of the F_{IS} values were negative except the one calculated for taxon CAS, and the taxa of San Lorenzo although none of the values were significantly different from zero. Neither the F_{IT} values calculated by taxon nor by locality were significantly different from zero. On the other hand, all of the F_{ST} values were positive and were significantly different from zero based on 95% CI.

Wright's F_{ST} statistics also reflected little genetic differentiation among populations in *C. argyrosperma* ssp. *sororia*, *C. argyrosperma* ssp. *argyrosperma*, *C. moschata*, and *C. pepo* (Table

4). The average F_{ST} value for all taxa was 0.111 (Table 4), and within-taxon mean values were 0.040 in *C. argyrosperma* ssp. *sororia*, 0.096 in *C. argyrosperma* ssp. *argyrosperma*, and 0.077 in *C. moschata*, with an overall average F_{ST} value for all 15 *Cucurbita* populations (without the outgroup) of 0.087 (Table 5). Thus, between 90.3% and 95.9% of the total allozyme variation in a taxon was found within populations.

Relatedness between populations-- Nei's genetic distance between populations of the three taxa ranged from 0.024 to 0.228 (Table 6). The range of mean genetic distances was lower between *C. argyrosperma* ssp. *argyrosperma* and *C. argyrosperma* ssp. *sororia* = 0.069, than between either subspecies and *C. moschata* (Table 6). Clustering of populations based on D shows that individuals of both subspecies of *C. argyrosperma* were interlaced and, with the exception of SL populations, appear to cluster more closely based on locality than on taxonomic identity (Fig. 2), while *C. moschata*'s populations form a clearly separate group with a well supported basal node (Fig. 2).

Levels of interpopulation gene flow-- The values of estimated gene flow for each locus were very variable and nearly all were greater than one (Table 4). The average N_em values averaged over all loci and taxa was 4.49, but ranged from 0.56 to 19.75 at different loci (Table 4). Within-taxon values were 5.74 for *C. argyrosperma* ssp. *argyrosperma*, 18.85 for *C. argyrosperma* ssp. *sororia*, and 11.54 in *C. moschata* (Table 5). N_em values averaged by locality ranged from 3.01 in Los Parajitos to 22.46 in San Miguel (Table 5). Average N_em values over loci within taxa and localities are high considering the variation among loci (Tables 4, 5).

DISCUSSION

Levels of genetic variation-- The per population average polymorphism ($\hat{p}$ = 0.99), allelic diversity (A = 2.5), and heterozygosity (H_e = 0.409) are high in comparison with values reported for other outcrossing, pollinator-pollinated, cultivated seed plants (p = 0.34; A = 2.67; H_e = 0.205; Hamrick and Godt, 1989, 1997), and are comparable to the highest values reported for cultivated selfing plants (p = 0.95; A = 1.34; H_e = 0.225; Jenczewski, Prosperi and Ronfort, 1999). Our estimates were also high compared with other *Cucurbita* studies. For example, Decker-Walters et al. (1990) found p = 19.3; A = 2.24; H_e = 0.068 for *C. pepo*, and p = 11.5; A = 1.43; H_e = 0.039 in *C. maxima*, even though other Cucurbitaceae taxa have higher H_e values (H_e = 0.225; Akimoto, Fukuhara and Kikuzawa, 1999). Nevertheless, we have to point out that our estimates may be upwardly biased because we used polymorphic loci previously reported in other studies.

Inbreeding-- The main genetic effects of inbreeding within populations is to increase the levels of homozygosity relative to those expected under conditions of random mating (Brown, 1979; Hamrick, 1989). Thus, comparisons of genotypic frequencies within populations with those expected under H-W equilibrium conditions can be used to detect historical levels of inbreeding or outcrossing in natural populations. In our *Cucurbita* populations, individual fixation index values showed significant departures from H-W in several cases and most values were slightly negative, indicating a small excess of heterozygotes and little inbreeding (mean F_{IS} = -0.071, not significantly different from 0). These results certainly do not rule out the possibility of occasional selfing, but they do argue against persistent selfing within these populations.

The combination of high genetic diversity within populations, little differentiation among populations, and high levels of gene flow indicate that these species are predominately outcrossing. Kirkpatrick and Wilson (1988) also found high outcrossing rates and low inbreeding in the cultivated *C. pepo* and in the weedy *C. texana* (Scheele) Gray [= *C. pepo* ssp. *overifera* (L.) Decker var. *texana* (Scheele) Filov].

Genetic differentation-- There was little genetic differentiation among the taxa. Nei's mean genetic distance values were low, ranging from 0.046 to 0.122. These distance values are lower than other *D* estimates for *Cucurbita* and within the range of values reported for conspecific seed plant populations (Decker, 1986; Decker and Wilson, 1987; Wilson, 1989; Merrick, 1991). For instance, in south-central Africa populations of *C. moschata* a study by Gwanama, Labuschagne, and Botha (2000) using RAPD markers reported *D* values from 0.31 to 0.41.

Our values of Wright's F_{ST} (average = 0.111) were lower than the reported average for animal-pollinated outcrossing seed plants (Hamrick, 1989) or for outcrossing cultivated seed plants (Hamrick and Godt, 1997) (F_{ST} mean values = 0.187 and 0.234, respectively). Both the *D* and the F_{ST} values suggest that there is substantial gene flow among populations of the three taxa we studied, as we will discuss later.

Our UPGMA analysis shows the existence of two major groups, *Cucurbita argyrosperma* (including both subspecies, *C. argyrosperma* ssp. *argyrosperma* and *C. argyrosperma* ssp. *sororia*) and *C. moschata*. However, we must caution that the bootstrap values in the tree are low, likely caused by the few differences between *Cucurbita* populations. In particular, populations of *C. argyrosperma* ssp. *argyrosperma* and *C. argyrosperma* ssp. *sororia* are more differentiated by locality than taxonomic identity, suggesting that their taxonomic classification should be reconsidered. However, it is possible that the similarity of these subspecies at allozyme loci does not reflect their differences at other loci determining morphological or reproductive characters important for species definition or that high levels of gene flow are eroding differentiation between them. This latter possibility is supported by the observation that the only population of *C. argyrosperma* that was clearly differentiated from another population was population CAA8 of *C. argyrosperma* ssp. *argyrosperma* collected from Tepec. This population occurs in a locality where the wild gourd, *C. argyrosperma* ssp. *sororia,* is not present. More genetic analyses and reproductive studies would be required before a firm conclusion could be reached about the taxonomic status of these two subspecies.

The distribution of allozyme variation in these three *Cucurbita* taxa includes high genetic variation within populations and low divergence among populations, consistent with the hypothesis that the taxa share a recent common ancestor and that there is ongoing gene flow between them. Our observation of homozygote deficiency in some populations could be due to high outcrossing rates or mating among genetically different individuals in large populations (disassortative mating) or because gene flow introduces variation into populations before random mating balances it. *Cucurbita argyrosperma* ssp. *sororia*, *C. argyrosperma* ssp. *argyrosperma* and *C. moschata* exist as extensive populations throughout the Jalisco region; they are often cultivated in adjacent plots, and the total number of individuals can range from a few hundred to several thousand. Given that several pollinating bee species move among the plants in the plots, visiting all *Cucurbita* taxa simultaneously (data not shown), high levels of interpopulation and intertaxa gene flow is not surprising.

Gene flow within wild-cultivated plant complex-- Gene flow can be an important evolutionary force. A small amount of gene flow is capable of counteracting other evolutionary forces such as mutation, drift, and selection (Slatkin, 1987). Previous studies have shown evidence of gene flow between populations of *C. pepo* and *C. texana* (Kirkpatrick and Wilson, 1988), and *C. argyrosperma* with *C. fraterna* L. H. Bailey (Wilson, Lira and Rodriguez, 1994). The low values of F_{ST} reported here suggest that there is exchange of genes among populations, even among those located several kilometers apart within the agroecosystem. Our very low F_{ST} estimates are lower than the average reported for outcrossing seed plants (Hamrick, 1989). In particular, we found high rates of gene flow in San Miguel among populations within 1.5 km of each other.

The great potential of pollen dispersal is likely to contribute to the high rates of gene flow among cultivated and weedy *Cucurbita* populations. Kirkpatrick and Wilson (1988) found outcrossing rates of 5 % in *C. pepo* and *C. texana*, at a distance of 1300 m. Arias and Rieseberg (1994) demonstrated gene dispersal from 2 to 7% at 800 m and 2% at 1000 m in the *Helianthus annuus* crop/weed complex. Jenczewski, Prosperi, and Ronfort (1999) reported evidence for the occurrence of crop/weed gene flow in *Medicago sativa*. In addition, in a separate study we found that 62% of farmers exchange squash seeds among themselves (Montes-Hernández, Merrick, and Eguiarte, in prep).

One might expect local farmers to keep *C. argyrosperma* ssp. *sororia*, *C. argyrosperma* ssp. *argyrosperma* and *C. moschata* distinct by selection of fruits and seeds to sow in the next season. However, it is possible that squash farmers could have difficulty in distinguishing among the taxa if one or more squash fruits are F_1 or part of the progeny of a cross between a landrace and a weedy type, particularly with *C. argyrosperma* ssp. *argyrosperma* (José Elizondo Anguiano, personal communication, squash farmer from El Chante).

Although genetic variation of many species could be reduced by human activities, some crop species have increased genetic variation due to certain selective practices inherent in their cultivation (Bye, 1993; Hernández, 1993). The continuous presence of wild populations of *C. argyrosperma* ssp. *sororia* and the milpa tradition of sowing together seeds of *C. argyrosperma* ssp. *argyrosperma* and *C. moschata* with corn adjacent to these wild populations probably both contribute to the high levels of gene flow observed here.

<u>Relevance in the light of release of genetic modified organisms</u>-- Gene flow between domesticated plants and their wild relatives may have two potentially harmful consequences, the evolution of increased weediness and the increased likelihood of extinction of wild relatives. Recent concern exists for the possibility of gene transfer between transgenic cultivated species and their wild relatives (Ellstrand, Prentice and Hancock, 1999). However, before this risk can be assessed, studies that examine the extent of introgression between the wild and transgenic plants are necessary (Payne, 1997). Our study suggests that crop to weed gene flow regularly occurs between wild and cultivated populations of squashes in Mexico. Mexico is the origin and center of diversity for *Cucurbita*, and we need to be extremely cautious in the field testing and wide-scale usage of transgenic plants of squash in Mexico.

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Table 1. Accessions of *Cucurbita* used in this study.

Number	Taxon	Status	Locality / municipality	Lat. N / Long. W	Elevation (m)
1	CAS	W	San Miguel, El Limón (SM)	19°51’07” / 104°04’54”	804
2	CAS	W	San Miguel, El Limón (SM)	19°51’07” / 104°04’54”	804
3	CAS	W	Los Parajitos, El Grullo (LP)	19°52’31” / 104°13’22”	996
4	CAS	W	El Chante, Autlán (EC)	19°43’09” / 104°12’24”	919
5	CAS	W	San Lorenzo, Ejutla (SL)	19°57’04” / 103°59’43”	1002
6	CAA	C	Los Parajitos, El Grullo (LP)	19°52’31” / 104°13’22”	996
7	CAA	C	San Lorenzo, Ejutla (SL)	19°57’04” / 103°59’43”	1002
8	CAA	C	Tepec, Amacueca (TE)	19°59’44” / 103°38’35”	1723
9	CAA	C	San Miguel, El Limón (SM)	19°51’07” / 104°04’54”	804
10	CAA	C	El Chante, Autlán (EC)	19°43’09” / 104°12’24”	919
11	CAA	C	La Cienega, El Limón (LC)	19°50’02” / 104°08’07”	847
12	CMO	C	San Miguel, El Limón (SM)	19°51’07” / 104°04’54”	804
13	CMO	C	Los Parajitos, El Grullo (LP)	19°52’31” / 104°13’22”	996
14	CMO	C	El Chante, Autlán (EC)	19°43’09” / 104°12’24”	919
15	CMO	C	San Lorenzo, Ejutla (SL)	19°57’04” / 103°59’43”	1002
16	CPE	C	Tepec, Amacueca (TE)	19°59’44” / 103°38’35”	1723

Note: Number, taxa [(CAS) = *C. argyrosperma* ssp. *sororia*, (CAA) = *C. argyrosperma* ssp. *argyrosperma*, (CMO) = *C. moschata*, and (CPE) = *C. pepo*], domestication status, [(W) = wild type and (C) = cultivated type], and their origin in the state of Jalisco, Mexico. Geographic coordinates, and elevation (m). Population numbers and taxon abbreviations that will be used to refer to the populations elsewhere in the text.

Table 2. Allele frequencies at 12 polymorphic loci for five *C. argyrosperma* ssp. *sororia* (numbers 1-5), six *C. argyrosperma* ssp. *argyrosperma* (numbers 6-11), four *C. moschata* (numbers 12-15) and one *C. pepo* (number 16) populations. For population names refer to Table 1.

		Population															
Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*Apx*1	1	0.37	0.44	0.45	0.64	0.32	0.26	0.57	0.45	0.58	0.73	0.72	0.38	0.47	0.91	0.47	0.90
	2	0.63	0.56	0.55	0.36	0.68	0.74	0.43	0.55	0.42	0.27	0.28	0.62	0.53	0.09	0.53	0.10
*Apx*2	1	0.57	0.51	0.39	0.59	0.51	0.50	0.59	0.46	0.61	0.61	0.44	0.50	0.65	0.63	0.42	0.28
	2	0.43	0.49	0.61	0.41	0.49	0.50	0.41	0.54	0.39	0.39	0.56	0.50	0.35	0.37	0.58	0.72
*Apx*4	1	0.21	0.23	0.27	0.70	0.35	0.28	0.38	0.42	0.45	0.55	0.30	0.38	0.44	0.59	0.32	1.00
	2	0.48	0.50	0.73	0.30	0.65	0.72	0.62	0.58	0.55	0.45	0.62	0.62	0.56	0.41	0.68	
	3	0.31	0.27									0.08					
*Pgi*1	1	0.13	0.06	0.24	0.14	0.17	0.29	0.04	0.05	0.10	0.15	0.03	0.41	0.09		0.13	0.50
	2	0.87	0.94	0.76	0.85	0.83	0.71	0.96	0.95	0.90	0.85	0.97	0.59	0.91	1.00	0.87	0.50
	3				0.01												
*Pgi*2	1	0.50	0.53	0.51	0.63	0.37	0.64	0.49	0.49	0.50	0.57	0.47	0.60	0.50	0.50	0.67	0.50
	2	0.50	0.47	0.49	0.37	0.63	0.36	0.51	0.51	0.50	0.43	0.53	0.40	0.50	0.50	0.33	0.50
*Lap*1	1	0.12	0.27	0.21	0.23	0.42	0.56	0.13	0.83	0.38	0.41	0.15	0.08	0.06	0.11	0.13	0.03
	2	0.81	0.59	0.79	0.77	0.58	0.44	0.61	0.17	0.62	0.59	0.85	0.46	0.50	0.89	0.51	0.07
	3	0.07	0.14					0.26					0.46	0.44		0.36	0.90
*Est*1	1	0.39	0.69	0.75	0.77	0.67	0.81	0.43	0.42	0.60	0.95	0.72	0.71	0.19	0.38	0.37	0.30
	2	0.61	0.31	0.25	0.23	0.33	0.19	0.57	0.58	0.40	0.05	0.28	0.28	0.81	0.62	0.63	0.70
	3												0.01				
*Me*1	1	0.44	0.37	0.51	0.40	0.49	0.45	0.47	0.35	0.53	0.44	0.55	0.39	0.33	0.27	0.35	0.41
	2	0.56	0.63	0.49	0.60	0.51	0.55	0.53	0.65	0.47	0.56	0.45	0.61	0.67	0.73	0.65	0.59
*Idh*1	1	0.41	0.49	0.43	0.53	0.57	0.83	0.42	0.25	0.56	0.08	0.28	0.34	0.35	0.33	0.61	0.48
	2	0.59	0.51	0.57	0.47	0.43	0.17	0.58	0.75	0.44	0.92	0.71	0.66	0.65	0.67	0.39	0.52
	3											0.01					
*Idh*2	1	0.33	0.37	0.44	0.56	0.39	0.31	0.09	0.14	0.20	0.15	0.39	0.60	0.65	0.61	0.49	0.37
	2	0.67	0.63	0.56	0.44	0.61	0.69	0.77	0.86	0.80	0.76	0.58	0.40	0.35	0.39	0.51	0.63
	3							0.14			0.09	0.03					
*Pgm*1	1	0.33	0.39	0.51	0.5	0.37	0.42	0.27	0.44	0.34	0.52	0.62	0.61	0.66	0.66	0.64	0.38
	2	0.67	0.61	0.49	0.5	0.63	0.58	0.73	0.56	0.66	0.48	0.38	0.39	0.34	0.34	0.36	0.62
*Mdh*1	1	0.27	0.39	0.51	0.38	0.47	0.55	0.22	0.28	0.40	0.23	0.38	0.54	0.47	0.57	0.51	0.15
	2	0.73	0.61	0.49	0.62	0.53	0.45	0.78	0.72	0.60	0.77	0.62	0.46	0.53	0.43	0.49	0.33
	3																0.52

Table 3. Levels of intrapopulation allozyme variation in four *Cucurbita* taxa.

Taxon	*N*	$\hat{p}$	*A*	*He*	*F*	Deviation from H-W Total no. loci	-	+
1. CAS	43	1.0	2.17	0.421 (0.092)	-0.142 ***	4	3	1
2. CAS	43	1.0	2.17	0.442 (0.114)	-0.152 ***	5	4	1
3. CAS	40.3	1.0	2.00	0.427 (0.055)	-0.151 ***	8	7	1
4. CAS	40	1.0	2.08	0.411 (0.068)	0.212 ***	5	1	4
5. CAS	42.6	1.0	2.00	0.433 (0.048)	0.268 ***	10	2	8
6. CAA	43.8	0.91	2.00	0.404 (0.065)	0.216 ***	11	3	8
7. CAA	37.3	0.91	2.17	0.406 (0.117)	-0.144 ***	3	2	1
8. CAA	43.3	0.91	2.00	0.374 (0.124)	-0.058 ns	5	2	3
9. CAA	39.3	1.0	2.00	0.419 (0.087)	-0.119 **	4	3	1
10. CAA	41.4	0.91	2.08	0.356 (0.133)	-0.072 ns	6	3	3
11. CAA	43	0.91	2.25	0.390 (0.129)	-0.102 **	4	3	1
12.CMO	40.8	1.0	2.17	0.457 (0.025)	-0.322 ***	8	7	1
13. CMO	42.3	1.0	2.08	0.415 (0.094)	-0.242 ***	7	6	1
14. CMO	37.9	0.91	1.92	0.356 (0.161)	-0.191 ***	5	4	1
15. CMO	37.3	1.0	2.08	0.437 (0.075)	0.061 ns	4	2	2
16. CPE	42.9	0.92	2.08	0.366	-0.215 ***	5	3	2

Note: The values given are means across 12 loci: effective mean number of alleles/locus (*A*), percentage of loci polymorphic ($\hat{p}$, a locus is considered polymorphic if the frequency of the most common allele did not exceed 0.95), mean expected heterozygosity (H_e; Nei's 1978 unbiased estimate with standard deviations in parentheses), mean fixation index (*F*). Significance of deviations of the Fixation indexes from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) was examined using X^2 test (Li and Horvitz, 1953). The number and result of tests for deviation of genotypic frequencies from those expected at Hardy-Weinberg (H-W) equilibrium are given, where Total No. loci = the number of loci out of 12 for which genotypic frequencies significantly differed from H-W, - = the number of loci with a significant excess of heterozygotes, and + = number of loci with a significant deficit of heterozygotes (Li and Horvitz, 1953). For taxon names refer to Table 1.

Table 4. Wright's (1965) F statistics for levels of gene flow within and among 16 populations of four taxa of *Cucurbita* (with jackknife means and standard deviation).

					Deviation from H-W po		
Locus	F_{IS}	F_{IT}	F_{ST}	N_em	Total No. populations	-	+
*Apx*1	-0.197 ***	-0.018	0.149 ***	1.25	7	6	1
*Apx*2	-0.281 ***	-0.251 ***	0.024 **	8.93	11	10	1
*Apx*4	0.121 ***	0.252 ***	0.149 ***	1.25	3	0	3
*Pgi*1	-0.379 ***	-0.182 ***	0.143 ***	1.32	4	4	0
*Pgi*2	-0.827 ***	-0.789 ***	0.021 *	10.24	16	16	0
*Lap*1	0.139 ***	0.382 ***	0.282 ***	0.56	9	2	7
*Est*1	0.197 ***	0.342 ***	0.181 ***	0.99	6	1	5
*Me*1	0.158 ***	0.167 ***	0.011	19.75	6	2	4
*Idh*1	-0.042	0.072	0.109 ***	1.79	7	5	2
*Idh*2	-0.055	0.054	0.104 ***	1.89	9	5	4
*Pgm*1	-0.009	0.047	0.056 ***	3.70	8	4	4
*Mdh*1	0.250 ***	0.316 ***	0.089 ***	2.25	8	0	8
Mean	-0.071	0.048	**0.111**	4.49			
SD	0.093	0.094	0.018	5.75			
95% CI	(-0.25, 0.07)	(-0.15, 0.17)	(0.05, 0.12)				

Note: The significance of the deviations of the F statistics from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) was tested using X^2 test (Li and Horvitz, 1953). The number and result of tests for deviation of genotypic frequencies from those expected at Hardy-Weinberg (H-W) equilibrium are given, where Total No. populations = the number of loci out of 12 for which genotypic frequencies significantly differed from H-W, - = the number of loci with a significant excess of heterozygotes, and + = number of loci with a significant deficit of heterozygotes (Li and Horvitz, 1953). Confidence intervals (95%, CI) for means of F statistics values are based on 1000 bootstrap samples. The value of F_{ST} for which the CI did not include zero is indicated in bold face.

Table 5. Wright's (1965) *F* statistics for levels of gene flow (Crow and Aoki's 1984, with standard deviation in parentheses) within and among populations of different number and taxa in *Cucurbita*, and localities. Confidence intervals (95%, CI) for means of *F* statistics values are based on 1000 bootstrap samples. Values for which the CI did not include zero are significant and are in bold face. For taxon names refer to Table 1.

Group	n	F_{IS}	F_{IT}	F_{ST}	N_em
Total	15	-0.061	0.031	**0.087**	5.22 (5.72)
SD		0.0939	0.0935	0.0188	
95% CI		(-0.25, 0.07)	(-0.15, 0.17)	(0.05, 0.12)	
Taxon					
CAS (W)	5	0.004	0.044	**0.040**	18.85 (40.20)
SD		0.1056	0.1058	0.0137	
95% CI		(-0.20, 0.19)	(-0.17, 0.22)	(0.02, 0.07)	
CAA (C)	6	-0.042	0.058	**0.096**	5.74 (7.69)
SD		0.1109	0.1118	0.0285	
95% CI		(-0.25, 0.14)	(-0.15, 0.24)	(0.05, 0.15)	
CMO (C)	4	-0.162	-0.073	**0.077**	11.54 (19.78)
SD		0.0995	0.0956	0.0259	
95% CI		(-0.34, 0.02)	(-0.25, 0.09)	(0.03, 0.13)	
Locality					
San Miguel	3	-0.142	-0.109	**0.028**	22.46 (35.09)
SD		0.1188	0.1206	0.0085	
95% CI		(-0.37, 0.07)	(-0.34, 0.10)	(0.01, 0.04)	
San Lorenzo	3	0.075	0.133	**0.062**	4.41 (5.19)
SD		0.0852	0.0822	0.0133	
95% CI		(-0.09, 0.22)	(-0.02, 0.28)	(0.04, 0.09)	
El Chante	3	-0.001	0.099	**0.099**	3.30 (3.24)
SD		0.1067	0.1133	0.0336	
95% CI		(-0.22, 0.19)	(-0.13, 0.29)	(0.04, 0.17)	
Los Parajitos	3	-0.062	0.061	**0.116**	3.01 (3.31)
SD		0.10	0.10	0.04	
95% CI		(-0.25, 0.11)	(-0.14, 0.23)	(0.05, 0.19)	

Table 6. Matrix of average Nei's (1978) genetic distance (*D* ; range in brackets) for all sampled populations of the four *Cucurbita* taxa, *C. argyrosperma* ssp. *sororia* (CAS), *C. argyrosperma* ssp. *argyrosperma* (CAA), *C. moschata* (CMO), and *C. pepo* (CPE).

	Taxon		
Taxon	CAS	CAA	CMO
CAS	0.0465 (0.024-0.084)		
CAA	0.069 (0.025-0.141)	0.0871 (0.027-0.161)	
CMO	0.085 (0.046-0.136)	0.122 (0.058-0.228)	0.0727 (0.035-0.129)
CPE	0.229 (0.249-0.328)	0.301 (0.235-0.385)	0.237 (0.221-0.263)

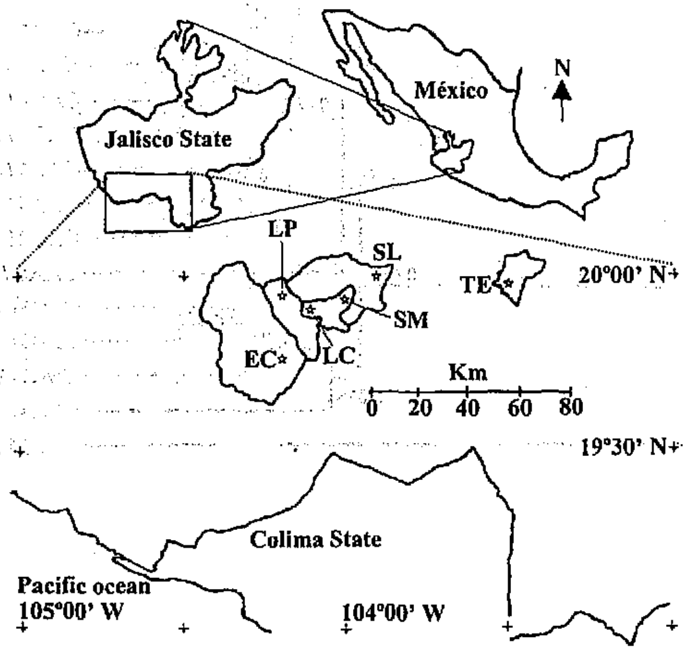

Fig. 1. Location of the six localities where the 16 *Cucurbita* populations were collected. For localities names refer to Table 1.

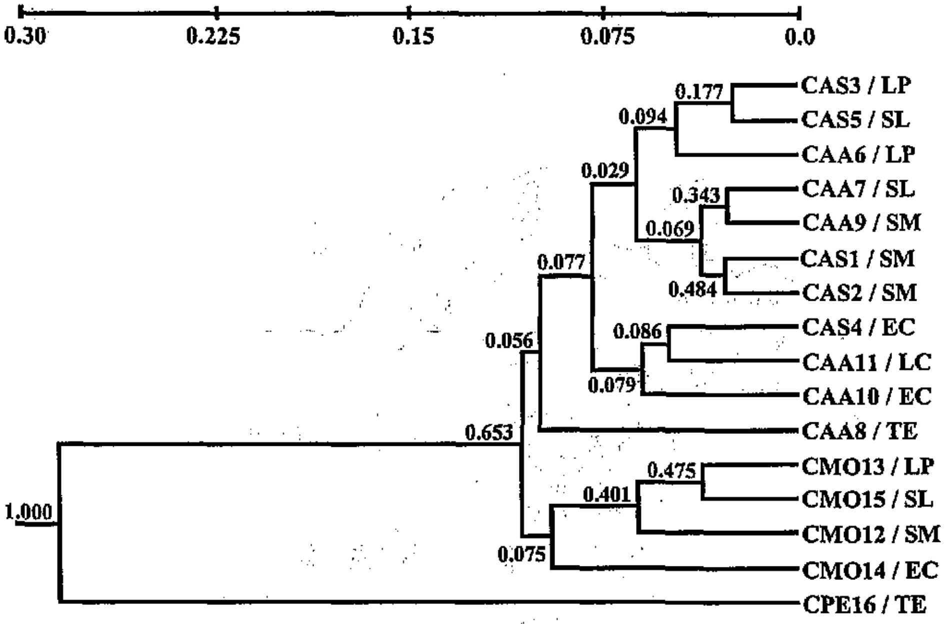

Fig. 2. Dendogram based on UPGMA of allozyme data for four taxa of *Cucurbita*: *C. argyrosperma* ssp. *sororia* (CAS), *C. argyrosperma* ssp. *argyrosperma* (CAA), *C. moschata* (CMO), and *C. pepo* (CPE), which was used as an outgroup. The numbers in the nodes show the proportion of similar replicates for 1000 bootstraps. For taxon and localities names refer to Table 1.

Capítulo V

DISCUSION GENERAL Y CONCLUSIONES

Las calabazas han sido un elemento básico en la alimentación y cultura del pueblo mexicano (Whitaker, Cutler y MacNeish, 1957; Cruces, 1987; Lira, 1995). En el capítulo 2 se muestran diferentes prácticas que realizan los campesinos sobre las calabazas en el SO del estado de Jalisco, que podrían contribuir a mantener la diversidad genética y la diferenciación de las calabazas que se documenta en el capítulo 4. El intercambio de semillas, a través del cual generalmente la nueva semilla obtenida se integra a la reserva de los agricultores destinada a la siembra, es una práctica común, la cual comparten con otros campesinos de la milpa en otras partes de México, y esta práctica contribuye a mantener la diversidad genética de los cultivos (Perales, 1998; Aguirre, 1999; Aguirre, Bellón y Smale, 2000). En la selección de frutos para la siembra, los agricultores toman en cuenta las características típicas de cada variedad local y, al parecer, esto contribuiría a explicar el mantenimiento de la identidad propia de sus cultivares locales aún frente al intenso flujo génico que se analizó en los capítulos 3 y 4. Además, esta práctica propicia que se conserve la base genética de estas especies en conjunto a nivel local o regional, como se ha reportado en el maíz y otros cultivos (Louette, Charrier y Berthaud, 1997; Perales, 1998; Brush y Meng, 1998; Louette y Smale, 2000). Los agricultores entrevistados en general manifestaron estar conscientes de la posibilidad de hibridación entre las calabazas cultivadas, así como entre éstas y el taxón silvestre. Por todo lo anterior consideramos que los agricultores, por medio de las diferentes prácticas culturales empíricas que realizan, contribuyen de manera preponderante en mantener la diversidad genética de las calabazas.

En el sistema milpa, el inicio de la floración fue coincidente en los tres taxa de estudio, con un ligero adelanto en *C. argyrosperma* spp. *argyrosperma* y *C. argyrosperma* spp. *sororia*, sugiriendo que el intercambio de polen entre ellas es posible. Las abejas especialistas en la polinización juegan un papel muy importante en la producción de frutos, tal y como lo han reportado diferentes autores para este género (Hurd, Linsley y Whitaker, 1971; Ordway et al., 1987; Robinson y Decker-Walters, 1997; Canto-Aguilar y Parra-Tabla, 2000). Asimismo, los polinizadores visitan en forma común a los tres taxa de *Cucurbita*, tal como fue reportado por Bautista (1997) en la misma región de estudio. Se encontraron pocas diferencias significativas en el número de visitas de los polinizadores entre los distintos taxa de *Cucurbita* estudiados. No obstante, la presencia de polvos fluorescentes entre los diferentes taxa presentó diferencias significativas entre los taxa, ya que hubo mayor presencia de ellos en *C. moschata*. Por lo anterior, el marcaje de polen y la presencia de polvos de un taxón en los otros dos, demuestra que el intercambio de polen entre los taxa de *Cucurbita* es una realidad, como había sido reportado en el mismo género (Kirkpatrick y Wilson, 1988), aunque en otras especies (*C pepo* y *C. texana* y localidades.

Al analizar la estructura y diversidad genética de poblaciones de los tres taxa de *Cucurbita* de la zona de estudio, se comprobó que los niveles de variación genética son altos y que el flujo génico entre los tres taxa de *Cucurbita* dentro de las localidades también es elevado. Asimismo, se confirmó una separación genética entre las poblaciones de *C. moschata* y las dos subespecies de *C. argyrosperma*, tal y como lo había señalado anteriormente Wilson (1989; 1990). Los valores encontrados de diversidad y diferenciación genética son altos al considerar otros valores promedio reportados para plantas de polinización abierta y mediada por insectos (Hamrick y Godt, 1989), y para plantas cultivadas con características similares a las calabazas (Hamrick y Godt, 1997).

Al conjuntar toda la información generada, es posible visualizar que las diversas prácticas de los agricultores, la actividad de los polinizadores y la biología floral de las plantas propician altos niveles de flujo génico, que contribuyen a mantener la diversidad genética de las calabazas de México. Bajo este escenario, las calabazas al igual que otros cultivos en sistemas tradicionales (Jarvis y Hodgkin, 1999), se ven favorecidas con la introgresión entre diferentes variantes, debido al manejo humano en conjunción con diversos factores bióticos, la cual ayuda a que se mantenga su diversidad genética.

Pero nos encontramos con un punto que también es de llamar la atención. La reducción tan drástica del número de agricultores que siembran calabaza, acentuada en los últimos seis años, la cual ha alcanzado al 66% de agricultores que la han dejado de cultivar, de la mitad del total registrado que han suspendido la siembra de este cultivo. De continuar esta tendencia puede traer consecuencias desastrosas sobre la diversidad genética de las calabazas en la zona de estudio, pues al reducirse la superficie de siembra y el número de agricultores que la cultivan se interrumpen los procesos que determinan el mantenimiento de la diversidad. Todo lo cual indica que se necesita poner atención en este problema e implementar medidas de conservación de la diversidad existente. De antemano creemos que es muy difícil que desaparezca el cultivo, por su gran arraigo cultural en la zona, tal y como se ha reportado en otros cultivos y regiones (Brush, 1991; Perales, 1998), pero sí es muy posible que se limite la diversidad hasta ahora presente, al reducirse el número de agricultores que las cultivan y con poca difusión hacia las nuevas generaciones de agricultores.

La estrategia que se propone para conservar este recurso, por considerarla más conveniente para poder llevar a cabo este fin, es el establecimiento de un programa de recolección de la diversidad local para conservarla *ex situ*. Esto lo proponemos ya que se considera que pensar en un programa de conservación *in situ* sería poco práctico, al estarse reduciendo el número de productores y la superficie cultivada de calabaza, por lo que la base genética a nivel regional se está limitando, y sería imposible mantener la misma dinámica existente hasta ahora en un programa de conservación de este tipo. Aunado a esto, se debe explorar la posibilidad de estimular una mayor producción, incrementado los usos hasta ahora reportados y buscar alternativas para darle mayor valor agregado a los poductos generados de las calabazas, como la industrialización de las semillas y frutos en general.

Por otro lado, toda esta información aporta bases para asegurar que la posibilidad de que se presente flujo génico entre las variedades transgénicas y sus parientes silvestres y con variedades locales de calabaza es muy grande, como había sido señalado por Wilson (1990), además de que se ha demostrado que es posible que esta hibridización suceda (Spencer y Snow, 2001). Por otro lado, se tiene el ejemplo reciente, de la posible contaminación por un transgene de variedades locales de maíz, en una zona de gran diversidad genética de maíz en México (Quist y Chapela, 2001). Aunado a esto, México es centro de origen y diversidad de calabazas cultivadas y silvestres, por lo que se debe evitar, en la medida de las posibilidades, las pruebas y uso a gran escala de plantas transgenicas de calabaza dentro de su territorio.

CONCLUSIONES

- Las prácticas de cultivar *C. moschata* y *C. argyrosperma* juntas, seleccionar los frutos que corresponden a cada una de sus variedades, así como intercambiar y seleccionar la semilla por parte de los agricultores, favorecen el flujo génico y la evolución de estos cultivos.

- La presencia y participación del taxón silvestre en la dinámica evolutiva de las calabazas integrantes de la milpa ayuda a incrementar la diversidad genética de las plantas cultivadas, lo cual es posible por el intercambio de genes entre los tres taxa.

- El uso de herbicidas para maíz, el incremento de la mano de obra con el uso de las calabazas en el maíz y el bajo precio de los frutos y semillas de las calabazas, han favorecido la disminución de la superficie de siembra de calabaza.

- El precio reducido del maíz ha contribuido a que se reduzca la superficie y número de agricultores que lo cultivan y por lo tanto también las calabazas, ya que en esta zona toda la calabaza se cultiva asociada al maíz.

- La disminución en el número de agricultores calabaceros y la superficie de siembra, además de que la mayoría de los agricultores milperos son ancianos, puede afectar la diversidad genética regional de este cultivo.

- El intercambio de semillas de calabaza para la siembra favorece la alta diversidad de las calabazas cultivadas (H_e = 0.407), al presentar flujo génico entre plantas de diferente origen, a pesar de que sean pocos frutos por parcela los que se seleccionan para la siembra.

- Al presentar una mayor coincidencia en el inicio de la floración entre *C. argyrosperma* spp. *argyrosperma* y *C. argyrosperma* spp. *sororia*, además de tener un parentesco más cercano, se presenta mayor flujo génico entre estas dos taxa. Los datos de genética de poblaciones apoyan este escenario.

- El periodo de apertura floral por día y la visita y forrajeo de los insectos son muy similares en los tres taxa de *Cucurbita*, lo que favorece que las mismas abejas visiten a los tres tipos de flores en tiempos similares. Por otro lado, sobresale la presencia de las abejas especialistas en la polinización de calabaza, principalmente *Peponapis azteca* Hurd y Linsley y *Xenoglossa gabii* Cresson, en el sistema milpa.

- El movimiento del polen es común entre los tres taxa de estudio y cerca del 50% del polen de cada taxón lo comparten con los otras dos taxa de *Cucurbita*.

- Los valores de F_{ST} y la diferencia genética entre poblaciones fueron bajos y, en consecuencia, la N_em mostró valores altos, lo que muestra en conjunto niveles elevados de flujo génico. Estos estadísticos variaron entre taxa y entre localidades.

- Se deben de emprender acciones tendientes a conservar *ex situ* la diversidad genética de estas plantas.

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Apéndice I. Factores que determinan la producción y flujo génico en el cultivo de calabaza (*Cucurbita* spp.) en el sistema milpa.

Encuesta Núm. ______________

I Datos del entrevistado

1.1 Nombre del entrevistado ______________________________ 1.2 Sexo (M/F)_______
1.3 Parentesco con el jefe de familia ________________________ 1.4 Edad (años) __________
1.5 Escolaridad (último grado de estudios) ______________________________________
1.6 Lugar ______________________ 1.7 Población ______________________________
1.8 Municipio ____________________________ 1.9 Estado _____________________
1.10 Latitud ______________ 1.11 Longitud ________________ 1.12 Altitud ______________
1.13 ¿Cuantas personas dependen económicamente de usted (incluyendo al productor) ____

1.14 ¿En los últimos tres años, que tan importantes han sido las siguientes actividades, en los ingresos de su familia y en que periodo las realiza?

Actividad	**Grado de importancia**	**Periodo en que realiza la actividad (meses)**
Producción de calabaza		
Producción de maíz		
Producción de otros		
Producción de ganado u otros animales		
Trabajo extrafinca en el pueblo		
Trabajos de artesanías		
Otros trabajos fuera de su comunidad, usted u otros miembros de su familia		
Apoyo de otros familiares que viven fuera de su comunidad en la república mexicana		
Trabajo no agrícola en la comunidad		
Apoyo de familiares que viven extranjero		

Nota: 1 = Muy importantes, 2 = Más o menos importantes, 3 = Poco o no importantes

1.15 ¿Cuántas personas más trabajan en su casa y contribuyen al gasto de la misma (1999)? ____
____

2 Tipo de propiedad en 1999

a. Privada ______________ ha
b. Ejidal ______________ ha
c. Comunal ______________ ha
d. Renta ______________ ha
e. Otras ______________ ha

3 Forma y textura del suelo de cada una de sus parcelas

Forma	Parcela 1	Parcela 2	Parcela 3	Parcela 4	Parcela 5
Plana					
Pendiente suave					
Pendiente fuerte					
Escarpada					
Otra					
Textura					
Arenosa					
Arcillosa					
Limosa					
Otra					

3.1 Tipo de suelo por parcela (nombre común empleado por el agricultor)

Parcela 1 ____

Parcela 2 ____

Parcela 3 ____

Parcela 4 ____

Parcela 5 ____

Otras ____

4 Uso actual de la tierra. Ciclo P-V 2000

Cultivo	Superficie	Fecha de siembra	Cultivo del ciclo anterior	Tipo de asociación	Producción total
Maíz					
Calabaza					
Caña					
Mezcal					
Pastos					

4.1 Sí no sembró calabaza este año, con su maíz, cuando fue la última vez, que sembró este cultivo ____________ año.

4.2 ¿Porqué razón dejo de sembrar calabazas en asociación (en caso de que alguna vez la haya sembrado)

____

____

____

____

____

4.3 Si nunca ha sembrado calabaza, asociada con maíz ¿Cuáles son las razones de que no realice esta asociación? ______

NOTA: A PARTIR DE AQUÍ SE REGISTRARA LA INFORMACION QUE SEA POSIBLE, AUNQUE SEA DEL ULTIMO AÑO, CUANDO SEMBRO CALABAZA.

4.4. Nombre común del (os) tipo (s) de calabaza (s) que siembra. ______

4.5 Si la calabaza se cultiva asociada, ¿Cuáles son los cultivos con los que se asocia?.

Cultivo	**Variedad del cultivo**	**Superficie**	**C.A.**	**C.M.**	**C.P.**	**C.F.**
Maíz						
Frijol						
Maíz-frijol						
Frutales						
Otros						
Otros						

C.A.= *C. argyrosperma*, C.M.= *C. moschata*, C.P.= *C.pepo*, C.F.= *C. ficifolia*

4.6 ¿Cuánta semilla de calabaza utiliza para la siembra?: ______ Kg/ha

______ Kg/ha

4.7 ¿Cuánta semilla del cultivo asociado (principal)? ______ Kg/ha

4.8 ¿Cuántos años lleva sembrando calabaza en forma continua? ______ años

4.8.1 ¿Porqué? ______

4.9 Distribución de semilla en el campo (revuelta, surcos, franjas, etc.) ______

4.9.1 Sí es en surcos intercalados. ¿Cada cuántos sucos la siembra? ______

4.9.2 Distancia entre matas ______ 4.5.3 Número de plantas por mata ______

4.10 ¿Siempre ha sembrado las mismas variedades? (S/N) ______

4.10.1 ¿Porqué? ______

4.11 Las variedades que siembra ahora, ¿Cuánto tiempo tiene sembrándolas? ______ años

4.11. 1¿Porqué? ______

4.12 Cuando se siembran dos o más tipos de calabaza juntas, modifica en algo su producción? S/N ________ 4.9.1 ¿Porque?____

____

____

4.12.2 ¿Se cruzan entre ellas? S/N ____________

4.12.3 ¿Como se sabe? ____

____

____

5. <u>Origen y manejo de semilla</u>

5.1 Para su siembra. ¿Ha comprado, intercambiado o le han regalado semilla de calabaza, sus familiares, vecinos o miembros de su comunidad o de otros pueblos? S/N ______________________

5.1.1 ¿Cuándo? ____

____

____

5.1.2 ¿Qué cantidad? ____

5.1.3 ¿Porqué? ____

____

____

____

5.2 Este año (último que haya sembrado). ¿Origen de la semilla de siembra de calabaza?.

Propio __________________ Otro familiar del mismo pueblo (grado) ______________________

Vecinos del mismo pueblo ______________ De otro pueblo (nombre) ______________________

Mercado del pueblo __________________ Mercado de la ciudad ________________________

Casa comercial (pueblo) ________________ Casa comercial (ciudad) ______________________

Otros ____

____

____

5.3 ¿Por qué utilizó este tipo de semilla? ____________________________

____

____

5.4 En caso de sembrar la semilla propia.

5.4.1 ¿Cuántos años tiene con ella? ____________________________________ años

5.4.2 Costo de la semilla ____ Kg/ha

5.5 ¿Recuerda usted algunos otros tipos de calabaza que se sembraban antes aquí (en su pueblo) y ya se perdieron? o ¿Qué se siembren en muy poca cantidad? S/N ___________________________

5.5.1 ¿Cómo le llamaban? ____

____

____

5.5.2 ¿Sabe usted porque se perdieron? o ¿Porqué se siembran muy poco? S/N______________

5.5.3 ¿Cuál es la razón? ____

____

____

6. Actividades agrícolas

6.1 En caso de emplear herbicidas para el control de malezas, en el cultivo principal, ¿Que tipo usa y cuando lo aplica? ______________________________

____

____

____

6.1.1 ¿Qué cantidad de herbicida aplica? ______________________________/ha
______________________________/ha
______________________________/ha

6.2 En caso de usar fertilizante, que tipo usa y en que cantidad.
Fertilizante químico ______________________________kg/ha
______________________________kg/ha
Estiércol ______________________________kg/ha
Abono verde ______________________________kg7ha
Otro ______________________________kg/ha

6.3 ¿Si su cultivo presenta plagas, como las controla y que cantidad emplea?
Control químico (producto y cantidad) ______________________________/ha
______________________________/ha
Control biológico (producto y cantidad) ______________________________/ha
Otros ______________________________/ha

6.4 ¿Si su cultivo presenta alguna enfermedad, como la controla y que cantidad emplea?
Control químico (producto y cantidad) ______________________________/ha
______________________________/ha
Control biológico (producto y cantidad) ______________________________/ha
Otros ______________________________/ha

7 Destino de la producción

7.1 *Fruto inmaduro o tierno*
7.1.1 Autoconsumo familiar ______________________________kg
7.1.2 Vendido dentro del pueblo
    Cantidad ______________________________kg
    Precio ______________________________$/kg
7.1.3 Vendido fuera del pueblo
    Cantidad ______________________________kg
    Precio ______________________________$/kg

7.2 *Fruto maduro*
7.2.1 Autoconsumo familiar ______________________________kg
7.2.2 Consumo animal (propios) ______________________________kg
7.2.3 Vendido dentro del pueblo
    Cantidad ______________________________kg
    Precio ______________________________$/kg
7.2.4 Vendido fuera del pueblo
    Cantidad ______________________________kg
    Precio ______________________________$/ha

7.3 *Semilla producto comercial*
7.3.1 Autoconsumo familiar ____________________ kg
7.3.2 Consumo animal (propios) ____________________ kg
7.3.3 Vendido dentro del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg
7.3.4 Vendido fuera del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg
7.4 *Semilla para siembra*
7.4.1 Uso propio ____________________ kg
7.4.2 Compartida (para regalar) ____________________ kg
7.4.3 Vendido dentro del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg
7.4.4 Vendido fuera del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg
7.5 *Otras partes de la planta (flor, tallo. etc.)*
7.5.1 Autoconsumo familiar ____________________ kg
7.5.2 Consumo animal (propios) ____________________ kg
7.5.3 Vendido dentro del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg
7.5.4 Vendido fuera del pueblo
    Cantidad ____________________ kg
    Precio ____________________ $/kg

7.6 Proporción o cantidad que representó la venta de calabaza (frutos o semillas) en relación con el cultivo principal. (1/2, 1/3, parte, etc.) ____________________

____

____

7.7 Si la cantidad anterior es importante, ¿Por qué mucha gente no siembra o ha dejado de producir calabaza? ____________________

____

____

8. Selección de frutos y semillas para sembrar
8.1 ¿Cuántos frutos de calabaza selecciona para sembrar el próximo ciclo (año) ____________
8.1.1 ¿Por qué ese número? ____________________

____

____

8.2 ¿Qué características debe presentar el fruto? ____________________

____

____

8.2.1 ¿Por qué? ____________________

8.3 ¿Qué características debe presentar la semilla? ____________________

8.3.1 ¿Por qué? ____________________

8.4 ¿Qué miembro de la familia se ocupa de realizar la selección de estos productos? ____________________

8.4.1 ¿Cuánto tiempo lleva realizando esta actividad? ____________________
8.4.2 ¿Por qué? ____________________

9 Actividades que realizan las mujeres en el cultivo de las calabazas

9.1 Selección de semilla para la siembra ____________________
9.2 Siembra de la calabaza ____________________
9.3 Cuidado de la planta ____________________
9.4 Extracción de semilla ____________________
9.5 Otras ____________________
9.6 Alguna de las características de las calabazas (fruto o semilla) que siembra han sido seleccionadas por sugerencia de su esposa o mamá S/N ________ 9.6.1 ¿Cuales? ____________________

9.6.2 ¿Por qué? ____________________

10. Alternativas de mejoramiento

10.1 Le gustaría trabajar dentro de su parcela, en conjunto con técnicos e investigadores, para obtener nuevas técnicas de mejoramiento y de producción de la calabaza? S/N ____________
10.1.1 ¿Por qué? ____________________

10.2 ¿Estaría dispuesto a probar nuevas variedades de calabaza que le suguiriera el técnico? S/N?

10.2.1 ¿Por qué? ____________________

10.3 ¿Cómo productor, que características le gustaría que tuviera una nueva variedad de calabaza que le presentara un técnico?

Más precoz ____________________

Tardía ____________________

Con mayor número de frutos ____________________

Frutos más grandes ____________________

Sabor más dulce del fruto ____________________

Color de la pulpa más fuerte ____________________

Más cantidad (número) de semillas ____________________

Menos semillas y más pulpa ____________________

Otras ____________________

____

____

____

11. <u>Plantas silvestres</u>

11.1 Existe alguna planta de calabaza silvestre, dentro o cerca de sus cultivos? S/N ____________

11.1.2 Nombre ____________________

11.1.3 Significado ____________________

11.2 ¿Tiene algún uso esa planta silvestre? S/N ____________________

11.2.1 ¿Cuáles? ____________________

____

____

11.3 ¿Se encuentra en su parcela esta planta (dentro o en las orillas) S/N ____________

11.3.1 ¿Desde cuando esta ahí esa planta? ____________________

11.4 ¿Su presencia es en todo el terreno? S/N ____________________

11.4.1 ¿Por qué? ____________________

____

11.5 ¿Elimina esta planta?. S/N ____________________

11.5.1 ¿Cómo lo hace? ____________________

11.6 ¿Ha observado que la calabaza que siembra es afectada cuando la silvestre está en el terreno? S/N

____

11.6.1 ¿Cómo se nota? ____________________

____

____

11.7 ¿Cree usted que se cruzan las calabazas que siembra, con las silvestres? S/N ____________

11.7.1 ¿Por qué? ____________________

____

____

11.7.2 ¿Es bueno o malo que se crucen? ____________________

11.7.3 ¿Por qué? ____________________

____

____

11.8 ¿Varía el inicio y época de floración de las calabazas cultivadas y silvestres? S/N ____________

11.8.2 ¿En que se nota? ____________________

____

____

11.9 Al ver una planta de calabaza, en su terreno o fuera de el, ¿Cómo se conoce que es una planta silvestre?. ¿Qué no se haya sembrado? ¿En qué son diferentes? (núm. de hojas, de flores, de frutos, semillas, etc.) ____

____

11.10 ¿Qué le gustaría que tuvieran sus calabazas que siembra, de lo que tienen las silvestres (de lo anterior) ____

____

____

11.11 ¿Es diferente la forma de las calabazas silvestres que crecen cerca del cultivo? S/N __________

11.11.1 ¿En que se nota? ____

____

____

11.11.3 ¿Por qué sucede esto? ____

____

Apéndice II. Padrón de agricultores que integraron la muestra a la cual se le aplicó la encuesta, en tres municipios de estudio, con el fin de describir el conocimiento empírico del cultivo de las calabazas y su relación con la conservación de la diversidad de este cultivo.

El Chante, mpio. de Autlán, Jal.	Municipio de Ejutla, Jal.	Municipio de El Limón, Jal.
Javier Cobían Pizano	Guadalupe Rincón Medina[A]	Adelelmo García Cobián[C]
Vicente Preciado Arias	Baldoviano Alvarado[A]	Rocio Lucero Michel Michel[C]
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Apéndice III. Recetas de buffers y tinciones de las isoenzimas.

BUFFER DE EXTRACCIÓN
Buffer YO más Buffer VEG II (3:1)

Buffer YO

Tris Acido Citrico	10 ml
Tris, free base	1.57 g
Ac. citrico	0.83 g
NADP (TPN)	0.05 g
NAD (DPN)	0.05 g
Acido ascórbico	0.018 g
EDTA	0.034 g
Suero Albumine bovino	0.1 g
2-Mercaptoetanol	0.33 ml

Aforar a 100 ml y ajustar pH7.0 con Acido citrico.

Buffer VEG II

Acido Bórico	0.31 g
Tergitol 17S9	2.0 ml
PEG 8000	2.0 g
PVP 40	7.0 g
PVP 360	1.0 g
Acido ascórbico	0.88 g
NAD (DPN)	0.02 g
Suero albumina bovino	0.1 g
Pyrdoxal 5-P	0.005 g
Sacarosa	0.27 g
Cisteina – HCL	0.19 g
2-Mercaptoetanol	0.66 ml

Aforar a 100 ml y ajustar pH7.1 con NAOH.

Sistema # 8 LiOH (Soltis et al., 1983)

Buffer del Gel (pH 7.6)

1) 0.042 M, Tris, free base	5.04 g
2) 0.007 M, Ac. Citrico:	
Monohidratado	1.47 g
Anhidro	1.35 g
3) 0.004 M, LiOH	0.16 g
4) 0.025 M, Ac. Bórico	1.56g

Aforar a 1000 ml y ajustar pH 7.6 con HCl, 1M.

Buffer del Electrodo (pH 8.0)

1) 0.039 M, LiOH	1.64 g
2) 0.263 M, Ac. Bórico	16.23 g

Aforar a 1000 ml y ajustar pH 8 con NaOH o HCl.

Sist. Histidina-Citrato (Cardy et al., 1980)

Buffer del Electrodo (pH 6.5)

0.065 M L-histidina	10.088 g
0.007 M, Ac. Citrico:	
Monohidratado	1.5 g
Anhidro	1.34 g

Aforar a 1000 ml y ajustar pH 6.5 con Ac. cítrico.

Buufer del Gel.

1 parte del Buffer del elctrodo y 3 partes de agua destilada.

EST, E.C. 3.1.1.1
(Esterasa)

- α-Naphtylacetate 1%	3.0 ml
- Agua destilada	40.0 ml
- Buffer de fosfatos pH 6.0	3.0 ml

Pesar Fast Blue RR Salt 75 mg

Dejar incubando a temperatura ambiente, por 60 min.

LAP E.C. 3.411.1)
(Leucina aminopeptidasa)

- Buffer 0.2 M Tris - Maleato , pH 5.2	50 ml
- L-Leucina β-naftilamida HCl 2.5%	1.0 ml

Pesar Fast Black K Salt 100 mg

Incubar en obscuridad a 37°C durante 30 min y posteriormente añadir el Fast Black K Salt, ya disuelto en 5 ml de agua destilada. Dejar incubar hasta que aparezcan las bandas.

MDH (EC. 1.1.1.37)
(Malato Deshidrogenasa)

- 0.05 M, Tris- HCl , pH 8.5	50 ml
- NAD (Nicotine adenine diphosphato)	1 ml
- 1 M DL-Malato pH 7.0	1 ml
- PMS 1%	0.4ml
- MTT o NBT 1%	1.0 ml

PGI (EC. 5.3.3.9)
(Fosfogluco isomerasa)

- 0.1 M, Tris- HCl , pH 7.5	50 ml
- Glucosa 6 fosfato deshidrogenasa (10u/ml)	3.0 ml
- 1.0 M, $MgCl_2$ (10%)	1.0 ml
- TPN (NADP) 1%	1.0 ml
- PMS 1%	0.5ml
- MTT 1%	1.0 ml

Pesar D Fructosa 6 fosfato, sal disódica 20 mg

Dejar incubando a 37°C hasta que aparezcan las bandas.

IDH (E.C.1.1.1.42)
(Isocitrato deshidrogenasa)

1) 0.2 M, Tris- HCl , pH 8.0	25 ml
- 1.0 M, $MgCl_2$ (10%)	1 ml
- TPN (NADP) 1%	1ml
- PMS 1%	0.2ml
- MTT 1%	0.5 ml

2) 0.2 M, Tris- HCl , pH 8.0 25 ml

Calentar hasta que hierva y se disuelva bien el agar, entonces mezclar 1) y 2).

Pesar:

- Ac. Isocitrico (Sal trisodica)	200 mg
- Agar	0.36 g

Dejar incubando a 37°C hasta que aparezcan las bandas.

ME E.C.1.1.1.40)
(Enzima Málica)

- 0.5 M, Tris- HCl , pH 8.0	40 ml
- 1 M DL-Malato pH 7.0	5 ml
- 1.0 M, $MgCl_2$ (10%)	1.0 ml
- TPN (NADP) 1%	1.0 ml
- PMS 1%	1.0 ml
- MTT 1%	0.2 ml

Incubar en obscuridad a 30°C, hasta que aparezcan las bandas.

APX (E.C. 1.1.1.7)
(Peroxidasa anódica, catódica)

Añadir:

- Dimetilformamida	7.0 ml
- Agua destilada	90 ml
- Peróxido de hodrogeno 3%	1.0 ml
- *Cloruro de calcio 1%*	*.0 ml*
- 1M Acetato de sodio pH 5.0	5.0 ml

Pesar 3-amino-9-ethilcarbazole 100 mg

Incubar a temperatura ambiente. Para la catódica usar la rebanada inferior.

PGM (E. C. 2.7.5.1)
(Fosfogluco mutasa)

- 1 M Tris-HCl, pH8	5.0 ml
- Agua destilada	40 ml
- 1.0 M, $MgCl_2$ (10%)	1.0 ml
- Glucosa 6 fosfato deshidrogenasa (10u/ml)	3.0 ml
- TPN (NADP) 1%	1.0 ml
- PMS 1%	0.3 ml
- MTT 1% (o NBT)	1.0 ml

Pesar α- D glucosa 1-fosfato 75 mg

Dejar incubando a 37°C hasta que aparezcan las bandas.