# UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

INSTITUTO DE ECOLOGIA

POSGRADO EN CIENCIAS BIOLOGICAS

## HISTORIA EVOLUTIVA DEL GENERO *ABIES* EN EL SUR DE MEXICO Y GUATEMALA: ESTRUCTURA GENETICA Y FLUJO GENICO.

# T E S I S

QUE PARA OBTENER EL GRADO ACADEMICO DE

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## RESUMEN

Se analizaron los efectos de la distribución espacial y de factores históricos como la fragmentación del hábitat y aislamiento poblacional sobre la estructura genética de las poblaciones de *Abies guatemalensis*, *A. religiosa*, *A. hickeli* y *A. flinckii*, especies distribuidas en el sur de México y Guatemala. Para realizar este análisis se utilizaron marcadores biparentales/nucleares, como las isoenzimas, y microsatélites de cloroplasto que poseen una herencia paterna en las Pinaceae. Se encontraron niveles relativamente bajos de diversidad genética para 16 loci isoenzimáticos (con un rango de $H_o$ promedio de 0.060 para *A. guatemalensis* a 0.113 para *A. flinckii*). En la mayoría de los casos se encontró un exceso de homócigos (con un rango de $F_{is}$ promedio de 0.074 en *A. flinckii* a 0.235 en *A. guatemalensis*) y niveles relativamente altos de diferenciación entre poblaciones, dentro de cada especie (con un rango de $\theta=F_{st}$ promedio de 0.073 en *A. hickeli* a 0.271 en *A. flinckii*). Los niveles de flujo génico encontrados fueron relativamente bajos (con un rango de *Nm* promedio de 0.672 en *A. flinckii* a 3.17 en *A. hickeli*). En el análisis de loci polimórficos de microsatélites de cloroplasto (cpSSRs) se detectaron 51 haplotipos diferentes, basándose en dos cpSSRs y se encontraron niveles altos de diversidad genética (con un rango de $H_e$ promedio de 0.75 para *A. flinckii* a 0.95 para *A. hickeli*). Se estimaron niveles relativamente altos de flujo génico (con un rango de *Nm* promedio de 3.32 para *A. guatemalensis* a 16.74 para *A. flinckii*).

La historia evolutiva de las especies estudiadas se analiza con los resultados obtenidos. Los datos sugieren que el polen puede ser el agente primario de flujo génico, que las especies de *Abies* estudiadas divirgieron recientemente y que, con la excepción de *A. flinckii,* aún no presentan una clara separación a nivel molecular.

## ABSTRACT

We analysed the effects of spatial distribution and historical factors such as habitat fragmentation and isolation of populations in the genetic structure of *Abies guatemalensis*, *A. religiosa*, *A. hickeli* and *A. flinckii*, species distributed in southern Mexico and Guatemala. Biparental/nuclear markers, such as isozyme markers and chloroplast markers, which are paternally inherited in Pinaceae were used in these analysis. Genetic diversity within populations was relatively low, measured for 16 isozyme loci (mean $H_o$ ranging from 0.060 for *A. guatemalensis* to 0.113 for *A. flinckii*). Populations in most cases had an excess of homozygosity (mean $F_{is}$ ranging from 0.074 in *A. flinckii* to 0.235 in *A. guatemalensis*) and high levels of among population differentiation within each species ($\theta=F_{st}$ ranging from 0.073 in *A. hickeli* to 0.271 in *A. flinckii*). Estimated levels of gene flow are relatively low (*Nm* ranging from 0.672 in *A. flinckii* to 3.17 en *A. hickeli*). In paternally inherited polymorphic chloroplast microsatellites (cpSSRs) a total of 51 haplotypes were detected based on two cpSSRs and high levels of diversity were found (mean $H_e$ ranging from 0.75 for *A. flinckii* to 0.95 for *A. hickeli*). Estimated levels of gene flow were relatively high (*Nm* ranging from 3.32 for *A. guatemalensis* to 16.74 for *A. flinckii*).

The evolutionary history of the species studied is analyzed in the light of the results. The data suggest that pollen might be the primary agent of gene flow, and that the *Abies* species studied are recently derived, and with the exception of *A. flinckii,* they are still not clearly differentiated at the molecular level.

# INTRODUCCIÓN

Actualmente se reconocen entre 39 y 55 especies de *Abies* en el mundo, (Rushforth 1989, Liu 1971, Farjon 1990, 1998), ocho de las cuales se encuentran en México, siendo seis de ellas endémicas. En el sur de México las especies de *Abies* se encuentran distribuidas en zonas de alta montaña, en poblaciones relativamente aisladas, separadas entre sí por varios kilómetros. Esta distribución parece estar influenciada por un periodo de calentamiento que siguió a la glaciación del Pleistoceno (Aguirre-Planter et al. 2000). Durante la última glaciación (~20 000 años antes del presente, AP) el clima en México era más frío (Lozano-García 1993), la temperatura era aproximadamente 6°C menor que en la actualidad (Vázquez-Selem y Heine 2004), lo que pudo favorecer la expansión de bosques de *Abies*. Existe evidencia paleobotánica y paleoclimática de que entre 12 500 y 9 000 años AP, al final del Pleistoceno, los bosques templados tuvieron su máximo desarrollo (Lozano-García 1993, Vázquez-Selem y Heine 2004). Por otro lado, Stute et al. (1995) encontraron evidencia fósil de que los límites del bosque templado estaban a unos 1000 m por debajo de su posición actual durante el último máximo glacial en el sureste de Estados Unidos. Esto sugiere que en México, durante la última glaciación, las especies templadas tuvieron una distribución más amplia que en el presente y las poblaciones de *Abies* pudieron haber estado en contacto (Aguirre-Planter et al. 2000). Posteriormente, después de 9000 años AP (incluyendo el presente), hubo un periodo de calentamiento y cuando la temperatura empezó a incrementarse la distribución de especies templadas se contrajo, ya que sus hábitats se hicieron más restringidos y fragmentados. Las poblaciones aisladas que encontramos en la actualidad pueden ser resultado de esta fragmentación, y probablemente muchas de ellas estuvieron conectadas entre sí en el pasado, lo que pudo haber influenciado significativamente la historia evolutiva y ecológica del género *Abies* en el sur de México.

**Objetivo de la tesis**

El objetivo general de esta tesis es analizar el efecto que tiene la distribución espacial de las especies y el efecto de factores históricos, como la fragmentación del

hábitat y el aislamiento de las poblaciones, en la estructura genética poblacional de *Abies guatemalensis, A. religiosa, A. hickeli y A. flinckii* con un marcador biparental, nuclear como las isoenzimas y con un marcador de herencia paterna, como el cloroplasto. Se analiza también la importancia del flujo génico en la evolución de estas especies y se proponen algunos lineamientos para su conservación.

Esta tesis se encuentra formada por cuatro secciones: Una introducción en donde se hace una revisión de la genética de poblaciones de distintas coníferas y particularmente de distintas formas para estimar el flujo génico. La segunda sección es un articulo publicado en American Journal of Botany acerca de la estructura genética de los *Abies* del sur de México y Guatemala; la tercera sección es un artículo que trata de la filogeografía de estas mismas especies. En la última sección se hace una discusión de los resultados obtenidos en ambos estudios, comparando ambos tipos de marcadores moleculares e infiriendo la posible historia evolutiva de las especies estudiadas y algunas pautas que deben de tomarse en cuenta para su conservación.

**Genética de poblaciones de coníferas**

Los patrones de variación y estructura genética de una especie están determinados por diferentes procesos evolutivos que actúan en las poblaciones, tales como la selección, la mutación, la deriva génica, migración o flujo génico y la recombinación mediada por el sistema reproductivo (Eguiarte 1990, Avise 1994). Además, la estructura genética de cualquier especie también está influenciada por factores biogeográficos, demográficos y eventos históricos, como las rutas de migración y de colonización o eventos como la fragmentación (Avise 1994). Por lo tanto, en el material genético de cada especie tenemos un importante archivo de su historia evolutiva, y analizando los niveles y patrones de la variación genética y su genealogía, se pueden inferir los patrones históricos y evolutivos que han dado origen a las especies y poblaciones actuales.

El impacto de la fragmentación en la estructura genética y el flujo génico dentro de especies con poblaciones aisladas es un tema de particular interés en biología evolutiva y es aún poco entendido (White et al. 2002). La fragmentación disminuye el

tamaño de una población e incrementa su aislamiento, y sus efectos en la dinámica del flujo génico y en el posible incremento en la endogamia pueden influenciar directamente la estructura genética de una especie (White et al. 2002).

Se ha encontrado que la mayoría de las coníferas estudiadas poseen niveles altos de variación isoenzimática (Hamrick et al. 1992, Fady and Conkle 1993, El-Kassaby and Ritland 1996, Ledig 1998; ver Tabla 1) y bajos niveles de diferenciación entre poblaciones (Ledig et al. 1997, Ledig 1998, Tabla 1). Hamrick et al. (1992) reportan para siete especies de *Abies* una heterocigosis promedio de 0.145 y una diferenciación genética ($G_{st}$) de 0.063. Para 28 especies de pinos de zonas templadas, Ledig (1998) encontró una $G_{st}$ promedio de 0.076, mientras que para ocho especies boreales y templadas de *Picea* el valor promedio de $G_{st}$ fue de 0.047 (Ledig et al. 1997). La baja diferenciación entre poblaciones encontrada se considera que se debe principalmente a su sistema reproductivo. El género *Abies*, así como otros géneros de la familia *Pinaceae*, son plantas monoicas (Martínez 1948, Liu 1971, Welch 1991) y el polen es dispersado por viento, potencialmente a grandes distancias (Llepelt et al. 2002). Así, los niveles de flujo génico en coníferas pueden ser altos, disminuyendo los niveles de diferenciación entre poblaciones. Sin embargo, estos estudios se han realizado en coníferas de zonas boreales y de zonas templadas, con una distribución continua y amplia y con poblaciones grandes. En México muchas especies de coníferas presentan poblaciones aisladas y fragmentadas, lo que indudablemente influye en los niveles y patrones de variación genética (Delgado et al. 1999; Aguirre-Planter et al. 2000, Molina-Freaner et al. 2001, Delgado et al. 2002)

Las poblaciones pequeñas y aisladas están por lo general sujetas a procesos como endogamia y pérdida de variación genética por deriva génica, y son especialmente susceptibles a la extinción (Frankham 1995, Keller y Waller 2002). La pérdida de heterocigosis, en combinación con un incremento en la expresión de alelos recesivos deletéreos, resulta en depresión por endogamia (Mitton 1992). Muchas coníferas sufren una intensa depresión por endogamia (Franklin 1970, Sorensen 1982) y se ha encontrado que en general los genotipos provenientes de cruzas endogámicas exhiben una menor tasa de germinación, menor tasa de crecimiento y mayor mortalidad (Ellstrand y Elam 1993).

Por otro lado, el tamaño efectivo y la distribución de las poblaciones pueden afectar significativamente los patrones y niveles de variación genética, y consecuentemente tener implicaciones importantes para la conservación. Así, si la mayor parte de la variación genética se encuentra dentro de poblaciones, los programas de manejo y las reservas que protejan algunas poblaciones o incluso una sola población grande, pueden ser suficientes para conservar la mayor parte de la diversidad de la especie. En contraste, si las poblaciones se encuentran muy diferenciadas genéticamente, entonces sería necesario proteger un mayor número de poblaciones (Eguiarte 1990).

**Flujo génico**

El flujo génico se refiere a todos los mecanismos que resultan en el movimiento de genes de una población a otra. Puede deberse al movimiento de gametos, semillas o individuos juveniles o adultos (en animales y en los casos de algunas plantas) y se puede referir a genes nucleares o genomas uniparentales como la mitocondria o el cloroplasto, o puede estar mediado por la extinción y recolonización de poblaciones enteras (Slatkin 1985a).

Anteriormente se pensaba que el flujo génico en general era muy restringido y de poca importancia evolutiva (Levin 1981). Sin embargo, al estimar el flujo génico en distintas especies se pudo observar que los niveles estimados generalmente son altos (Riesberg y Burke 2001) y que pueden actuar como una fuerza que mantiene integrada a la especie. Los patrones y niveles de flujo génico determinan hasta que grado cada población de una especie es una unidad evolutiva independiente (Slatkin 1994). Si el flujo génico entre poblaciones de una especie es alto, entonces todas las poblaciones evolucionan de manera conjunta (en ausencia de selección), pero si es muy bajo, las poblaciones de una especie pueden divergir y evolucionar casi independientemente. Si continúa la diferenciación puede llegar a surgir aislamiento reproductivo, y en consecuencia, el establecimiento de linajes evolutivamente independientes.

Se han utilizado distintos métodos para estimar los niveles de flujo génico. Los métodos indirectos se basan principalmente en la observación de la distribución espacial de alelos en las poblaciones, utilizando las frecuencias alélicas obtenidas de muestras de distintas poblaciones y de esta manera se hacen inferencias sobre los

niveles o patrones de flujo génico entre las poblaciones (Slatkin 1985a). La mayoría de los modelos teóricos de flujo génico surgen de los conceptos desarrollados por Sewall Wright, basados en poblaciones continuas y utilizando un enfoque de aislamiento por distancia o en poblaciones como islas que se diferencian por mutación y deriva génica (Wright 1943). El modelo usado comúnmente para estimar flujo génico es el modelo de islas infinitas (infinite islands model) de Wright (1951). Este modelo considera condiciones en equilibrio entre un número infinito de islas o subpoblaciones de igual tamaño, que intercambian migrantes entre cualquiera de las islas con igual probabilidad, a una tasa constante. Las poblaciones pueden ser tratadas como réplicas, y el modelo se puede caracterizar con sólo dos parámetros: tamaño poblacional ($N$) y tasa de migración ($m$). La importancia de la deriva génica es proporcional a $1/N$, mientras que la importancia del flujo génico es proporcional a $m$ (Slatkin 1985a).

Otro modelo es el de "stepping-stone", introducido por Kimura (1953). En este modelo las poblaciones se localizan en una especie de enrejado de una, dos o tres dimensiones y los individuos sólo pueden moverse entre poblaciones adyacentes.

Wright (1951) introdujo un método para dividir el coeficiente de endogamia en una población subdividida en un componente debido a apareamientos no aleatorios dentro de poblaciones ($F_{is}$) y otro componente debido a la subdivisión entre poblaciones ($F_{st}$). $F_{st}$ es definido como:

$$F_{st} = (H_T - H_S)/H_T ,$$

en donde $H_T$ es el promedio de la heterocigosis esperada en la población total, para todos los loci, y $H_S$ es el promedio de la heterocigosis esperada dentro de subpoblaciones para todos los loci.

$F_{st}$ mide la reducción en la heterocigosis debida a diferenciación genética entre poblaciones y se relaciona con la migración de la siguiente manera:

$$F_{st} \approx 1/ (4\ Nm + 1)$$

$F_{st}$ se estima mediante datos aloenzimáticos o moleculares y, el flujo génico efectivo puede posteriormente calcularse en el equilibrio como:

$$Nm \approx \frac{1}{4} (1/F_{st} - 1)$$

Bajo este modelo, de acuerdo con Wright (1969), una tasa de migración >1 en cada generación es suficiente para contrarrestar la diferenciación genética debida a deriva génica.

Crow y Aoki (1984) propusieron un modelo poblacional con un número finito de islas (modelo de n-islas), en el que el equivalente para alelos múltiples de $F_{st}$ es el parámetro $G_{st}$ de Nei (Nei 1973), definido como:

$$G_{st} \approx 1/ (4Nm\alpha + 1)$$

Donde $\alpha = [n/ (n-1)]^2$, y $n$ es el número de subpoblaciones

Este modelo asume que las poblaciones están en equilibrio y que las islas intercambian migrantes entre si con igual probabilidad a una tasa constante.

Otro método para estimar *Nm* es el de "alelos privados" (Slatkin 1985b,1981), mediante el cual se obtienen muestras de distintas localidades y se obtiene p(1), que es la frecuencia promedio de alelos que sólo están en una muestra. Slatkin (1985b) encontró que para el modelo de islas y el de "stepping stone",

$\log_{10}[p(1)]$ está linealmente relacionado con $\log_{10}(Nm)$, entonces

$$\log10[p(1)] = a\log10(Nm) + b$$

donde p(1) es la frecuencia promedio de los alelos privados y *a* y *b* son constantes determinadas por datos simulados y dependen del número de individuos muestreados en cada subpoblación (ver Slatkin, 1985b). Este método y la estimación de *Nm* a partir de $F_{st}$ son parecidos, ya que ambos se basan en analizar la dispersión de la distribución de las frecuencias alélicas entre poblaciones. Sin embargo, simulaciones han demostrado que el método de alelos privados es más sensible a errores en la colección de datos (Slatkin 1994).

Otro modelo relacionado con el flujo génico es el de "aislamiento por distancia", que examina la relación del flujo génico entre pares de poblaciones (*M*, equivalente a *Nm* entre pares de poblaciones) con la distancia geográfica (Slatkin, 1993). Bajo este modelo, se estiman valores de *M* a partir de valores para pares de poblaciones de $\theta$ (que es una medida de diferenciación entre poblaciones, Weir y Cockerham 1984) y Slatkin propone analizar con una regresión de log10*M* en log10*k* (*k*= distancia geográfica). Para determinar si existe una relación lineal significativa entre el flujo génico y la distancia entre poblaciones se utiliza comúnmente una prueba de Mantel (1967), ya que los valores de M para los diferentes pares de poblaciones no son independientes.

Una crítica común a la estimación de flujo génico basada en estimadores que utilizan la variación en las frecuencias alélicas, como $F_{st}$ es que supone un modelo de estructura poblacional de islas infinitas que es poco real, ya que considera un número infinito de poblaciones, donde hay ausencia de selección o mutación, que el flujo génico no está afectado por la distancia geográfica entre poblaciones y que cada población está en un equilibrio entre migración y deriva (Slatkin y Barton 1989; Niegel 2002).

Slatkin y Barton (1989) revisaron la relación entre $F_{st}$ y flujo génico, tanto teóricamente como mediante simulaciones, encontrando evidencia para apoyar el uso del modelo de islas. Estos autores concluyeron que en una población subdividida que está en equilibrio demográfico, tanto el método de $F_{st}$ como el de alelos privados proveen estimados razonablemente correctos de *Nm* bajo una variedad de condiciones.

Por otro lado, Whitlock y McCauley (1999) proponen que existe evidencia de que muchos de los loci a partir de los cuales se estima $F_{st}$ están sujetos a selección, y este factor puede tanto sobreestimar como subestimar los valores de $F_{st}$. Particularmente cuando la tasa de migración es muy baja (y en ausencia de deriva génica), la selección puede dominar el patrón de diferenciación. El ligamiento a alelos sujetos a selección local puede aumentar la $F_{st}$. Finalmente, la selección causada por depresión por endogamia puede sobrestimar la estimación de la migración (Whitlock y McCauley 1999). Sin embargo, Slatkin y Barton (1989) encontraron que la $F_{st}$ en el modelo de islas no es muy sensible a la selección que favorece a uno o dos alelos, sólo es

importante si diferentes alelos se encuentran favorecidos en distintas localidades geográficas.

La tasa de mutación también puede afectar los patrones de diferenciación genética entre poblaciones. Aunque se propone que $F_{st}$ no es muy sensible a las tasas de mutación, ésto sólo se cumple si los tamaños poblacionales son pequeños (de 1000 o menores) y las tasas de mutación son mucho menores que las tasas de migración (Crow y Aoki, 1984). En marcadores como microsatélites y secuencias de mitocondria, las tasas de mutación pueden ser muy altas, lo que puede disminuir $F_{st}$ y determinar una sobreestimación de *Nm* (Hedrick 1999). Slatkin (1991) definió $F_{st}$ en términos de tiempos de coalescencia entre alelos y desarrolló otro estimado, $R_{st}$, como un estimador de $F_{st}$ que es relativamente insensible a tasas de mutación y más apropiado para loci que mutan en forma de paso a paso ("step-wise"). Sin embargo, $R_{st}$ tiene una mayor varianza que otras medidas de diferenciación entre subpoblaciones, y es muy sensible a violaciones del modelo de mutación paso a paso (Gaggiotti et al. 1999) y, por lo tanto, no es claro que en la práctica ofrezca ventajas sobre $F_{st}$, aún en marcadores que teóricamente se ajustan al modelo de mutación paso a paso ("step-wise").

Otro problema en relación a la estimación de $F_{st}$ , es el del equilibrio. El tiempo en generaciones requerido para que $F_{st}$ esté cerca del equilibrio es $1/[2m+1/(2N)]$, donde $m$ es la tasa de migración y $N$ es el tamaño poblacional (Crow y Aoki 1984). Cuando no hay flujo génico, el tiempo requerido sería igual a $2N$. Si existieron poblaciones grandes que estuvieron históricamente en contacto y que actualmente se encuentran aisladas, estarían fijadas para distintos alelos en el equilibrio, pero el tiempo requerido para aproximarse a ese equilibrio podría ser mayor a la edad de la mayoría de estas especies. Estos estimados darían estimaciones de flujo génico diferentes de cero en especies que han estado aisladas reproductivamente durante mucho tiempo (Niegel 2002).

El modelo de islas es una abstracción conveniente que aisla los efectos opuestos de deriva génica y flujo génico, y aunque $F_{st}$ no siempre da estimaciones "exactas" de flujo génico, es poco probable que estos estimados estén sobrestimados por varios órdenes de magnitud (Niegel 2002). Niegel (2002) propone que el número de poblaciones no tiene que ser infinito, ni necesariamente muy grande para que $F_{st}$ de

una estimación acertada de *Nm*, y que la mutación y la selección sólo son importantes cuando las poblaciones son muy grandes. Niegel (2002) también demuestra que aunque el flujo génico esté limitado por distancia, se espera que el valor total de $F_{st}$ sea similar al obtenido por el modelo de islas (Slatkin y Barton 1989). El problema del uso de $F_{st}$ surge cuando la deriva génica es un proceso débil (los tamaños efectivos de las poblaciones son grandes). $F_{st}$ puede pensarse como un balance entre deriva génica y flujo génico y valores muy pequeños de flujo génico (<<1) pueden acercar $F_{st}$ hacia 0.

En conclusión, los datos y simulaciones indican que el uso de $F_{st}$ para estimar *Nm* es útil y sencillo. Los métodos indirectos tienen la ventaja de que pueden incorporar los efectos de todos los componentes históricos de la dispersión y se puede obtener un promedio de la variación en la dispersión a través del tiempo. Finalmente, los niveles de flujo génico estimados con $F_{st}$ reflejan flujo génico histórico, no el flujo génico que está ocurriendo en el presente.

**Métodos genealógicos**

La distribución de la variación genética en poblaciones de plantas está fuertemente influenciada tanto por las fuerzas microevolutivas que están actuando, como son el flujo génico y la selección, así como también por la historia filogenética de las poblaciones y especies (Schaal et al. 2003). El uso de estadísticos tradicionales para determinar la estructura de las poblaciones, como los estadísticos F de Wright y otros métodos indirectos que utilizan las frecuencias alélicas, los cuales se mencionaron anteriormente, no toman en cuenta la información de las relaciones evolutivas de los variantes genéticos y por lo tanto no es posible distinguir entre efectos históricos y procesos recurrentes.

Muchas secuencias de genes proveen de una alta variación molecular que es neutral y que puede ser convertida a una genealogía. En este contexto, el uso de secuencias de ADN para inferir procesos evolutivos en el pasado a nivel poblacional, ha sido enriquecido por el concepto de filogeografía propuesto por Avise (1994). La filogeografía examina la distribución de la genealogía de alelos o haplotipos en un contexto geográfico, utiliza la información histórica contenida en los genes y caracteriza

la subdivisión de poblaciones al reconocer patrones geográficos de la estructura genealógica a lo largo del rango de distribución de una especie (Avise 1994).

A nivel poblacional es preferible el uso de redes de haplotipos en lugar de árboles filogenéticos, ya que la inferencia cladística de relaciones filogenéticas requiere que las variantes genéticas no formen linajes reticulados (Schaal et al. 1998). Las genealogías trazan las relaciones evolutivas entre haplotipos o alelos (Schaal et al. 2003) y con esta información se pueden reconstruir los procesos evolutivos con los principios de la coalescencia. Los métodos que utilizan estos principios tratan de modelar el pasado utilizando un proceso estocástico, la coalescencia, la cual se basa en el concepto de que las secuencias alélicas, o alelos en una población pueden ser rastreados hacia atrás en el tiempo hasta el punto en el que coalescen a la secuencia o alelo ancestral más reciente. Los patrones de coalescencia generalmente se representan usando árboles de genes que muestran la genealogía de los alelos en la población.

La idea principal detrás de la coalescencia es que, en ausencia de selección, los linajes muestreados pueden ser vistos hacia atrás en el tiempo "escogiendo" al azar a sus padres y cuando dos linajes escogen al mismo padre hay un evento de coalescencia. Eventualmente todos los linajes coalescen a un mismo y único linaje, por ejemplo, el ancestro común más reciente (Rosenberg y Nordborg 2002). Los métodos genealógicos son utilizados para estimar los parámetros del proceso genealógico que da como resultado a cada árbol. El proceso de coalescencia es una herramienta matemática poderosa que puede ser utilizada para estimar parámetros poblacionales como las tasas de mutación o migración y otros procesos (Rosenberg y Nordborg 2002).

Templeton et al. (1987, 1995) y Templeton (1998) han propuesto un análisis basado en los principios de coalescencia para conocer las relaciones entre poblaciones, que es el análisis de clados anidados. Bajo un contexto geográfico es posible obtener información de procesos históricos, como estimaciones más cercanas y más realistas de flujo génico, de la deriva génica o de la expansión, además de ayudar a estimar parámetros más complejos y modelos reales de estructura poblacional. Avise (2000) propone que los cambios en tamaños poblacionales o en niveles de flujo génico a través del tiempo dejan patrones que pueden reconocerse y estos patrones son

marcas de historias poblacionales particulares. Por ejemplo, se predice que las genealogías van a presentar una filogenia en forma de estrella en una población que se ha estado expandiendo (Avise 2000). En el análisis de clados anidados (NCPA, por las siglas en inglés, nested clade phylogeographical analisys) de Templeton et al. (1995), se reconstruyen redes de haplotipos que se unen en clados anidados con el programa TCS (Phylogenetic network estimation using statistical parsimony, Clement et al. 2000). Este algoritmo de anidamiento (Templeton et al. 1987) agrupa haplotipos y después clados, dependiendo del número de cambios mutacionales, separándolos paso a paso hasta que todos los haplotipos/clados queden agrupados y unidos en grupos de uno, dos o más pasos mutacionales, donde el último nivel de anidamiento comprende la red entera (Templeton et al. 1987). La relación entre haplotipos/clados y la geografía se puede examinar a través de un análisis estadístico llamado de "permutaciones", utilizando por ejemplo el programa GEODIS (A tool to perform the NCPA, Posada et al. 2000). Este análisis permite evaluar si existen asociaciones significativas entre la ubicación geográfica y las posiciones del haplotipo o del clado. En clados en los que hay una asociación significativa, se puede hacer un análisis de distancia, en la cual se comparan las distancias del clado $D_c$, que mide el rango geográfico de un clado en particular y la distancia del clado anidado $D_n$, que mide como un clado en particular se distribuye geográficamente en relación a sus clados evolutivamente más cercanos, clados hermanos ( clados en la misma categoría de anidamiento). En particular, la distancia del clado mide la distancia promedio que existe entre un individuo que posee un haplotipo del clado de interés y el centro geográfico de todos los individuos que poseen haplotipos del mismo clado. La distancia del clado anidado mide la distancia promedio que existe entre un individuo que posee un haplotipo del clado de interés y el centro geográfico de todos los individuos que poseen haplotipos del siguiente nivel de anidamiento, que contiene al clado de interés (Templeton 1998). Los contrastes entre estas medidas de distancia entre clados externos (clados que no son nodos interiores en la red de haplotipos) y clados internos inmediatos son importantes para discriminar las causas potenciales de la estructuración geográfica de la variación genética (Templeton et al. 1995, Templeton 1998). Para determinar si las distintas medidas de distancia son significativas se realiza un análisis de permutaciones, el cual simula la hipótesis nula de una distribución geográfica al azar para todos los clados dentro de

una categoría o nivel de anidamiento (Templeton 1998). Los resultados se interpretan utilizando la clave de inferencias de Templeton (2004). Esta clave de inferencias proporciona una serie de criterios que permiten discriminar entre el papel que han jugado los eventos de flujo génico, el aislamiento por distancia, la expansión y los eventos de colonización a larga distancia en los procesos históricos que resultan en las relaciones observadas entre haplotipos/clados.

**Especies de estudio**

Farjon (1990, 1998) reconoce la existencia de 46 especies de *Abies* en el mundo, distribuidas en el hemisferio Norte aunque Liu (1971) considera que los taxa se agrupan en 39 y Rushforth (1987) propone hasta 55 especies. Se han propuesto varias clasificaciones para el género *Abies*, con base en diferentes características morfológicas (Martínez 1948, Liu 1971, Rushforth 1989, Farjon 1990, 1998). Martínez (1948) reporta ocho especies para México, seis de ellas endémicas, mientras que bajo su sistema de clasificación Liu (1971) considera que son seis las especies mexicanas, cuatro de ellas endémicas. De la misma manera, Farjon (1990, 1998) propone la existencia de seis especies en México, considerando a cuatro de ellas endémicas, mientras que Rushforth (1989) describió dos especies adicionales del oeste de México y Debreczy y Racz (1995) propusieron tres más.

Según la clasificación de Farjon (1990,1998), basada en características morfológicas (Tabla 2), los *Abies* distribuidos en México pertenecen a dos secciones:
Sección *Grandis*. Engelm. Emend. Farjon et Rushforth. En esta sección se agrupan *A. concolor* (Gord. et Glend.) Lindl., *A. durangensis* Mart., *A. guatemalensis* Rehder y también *A. grandis* (Dougl.) Lindl., que no es una especie que se encuentre distribuida en México.
Sección *Oiamel* Franco, la cual se divide en:
Subsección *Religioseae* (Matzenko) Farjon et Rushforth, en la cual se encuentran agrupadas las especies *A. religiosa* (H. B. K.) Schl. et. Cham. y *A. vejarii* Mart.
Subsección *Hickelianae* Farjon et Rushforth se encuentra *A. hickeli* Flous et. Gaussen.

En este trabajo se consideraron cuatro especies de *Abies* distribuidas en el sur de México y Guatemala, las cuales presentan distribuciones contrastantes (Tabla 3,

Figura 1). *Abies guatemalensis* (Figura 2), es la especie con la distribución más sureña, distribuida entre 2000 y 4000m de altitud en Guatemala y en los estados de Chiapas, Oaxaca, Guerrero, Jalisco, Hidalgo y San Luis Potosí en México (Martínez 1948, Donahue et al. 1985). Aunque tiene una distribución amplia, sus poblaciones están aisladas, formando manchones separados por grandes distancias. Esta especie era considerada común hasta 1940, pero se ha explotado ampliamente a lo largo de todo su rango de distribución y ahora se le considera en peligro de extinción. Su madera es muy preciada por su suavidad y se utiliza localmente como leña y carbón y como material para construcción. Sus árboles han sido intensamente talados, ya que esta especie crece en suelos profundos y fértiles de montaña, que son adecuados para la agricultura y el pastisaje. Se encuentra incluida en la NOM-ECOL-059-1994 como en peligro de extinción, también en la lista roja de plantas de la IUCN y en el apéndice 1 del CITES. Su comercio está prohibido en México y Guatemala.

*Abies religiosa* (Figura 3) está distribuida principalmente por arriba de los 2000m a lo largo del eje volcánico transversal, en los estados de Guerrero, Jalisco, Michoacán, México, Morelos, Hidalgo, Tlaxcala, Puebla y Veracruz, así como también en el Distrito Federal (Martínez 1948). Tiene una distribución más continua que *A. guatemalensis*. Posee un uso local artesanal, se emplea también para la elaboración de balsámicos y los árboles pequeños son utilizados como árboles de navidad. En algunas localidades son cultivados con este propósito y así como para la producción de madera.

*A. religiosa* var. *emarginata* Martínez se distribuye en los estados de Jalisco y Michoacán, y *A. guatemalensis* var. *jaliscana* Martínez tiene una distribución muy restringida al oeste de Jalisco. Rushforth (1989) consideró a estas dos variedades formando parte de una nueva especie, *A. flinckii* Rushforth. Observaciones de campo sugieren que estas variedades son morfológicamente similares y distintas de *A. religiosa* y *A. guatemalensis*. Difieren de estas últimas especies también en su fenología, empezando su crecimiento vegetativo hasta tres meses antes. En la Sierra de Manantlán árboles de *A. religiosa* y *A. flinckii* se encuentran entremezclados en el mismo sitio, manteniéndose sus diferencias morfológicas y fenológicas, lo que sugiere que se trata de especies diferentes, aisladas reproductivamente. Actualmente *A. flinckii* está considerada en la NOM-ECOL-059-1994 como especie que requiere protección especial.

*Abies hickeli* (Figura 4) tiene una distribución limitada, con sólo algunas poblaciones en los estados de Oaxaca y Veracruz (Martínez 1948). Su madera se emplea para construir techos de casas (tejamanil) en regiones cercanas a donde crece. También se utiliza para la elaboración de muebles y los árboles pequeños se utilizan como árboles navideños. Actualmente se encuentra incluida en la NOM-ECOL-059-1994 como en peligro de extinción.

Tabla 1. Número de poblaciones (*N* Pob), número de loci analizados (*N* loci), distribución, heterocigosis esperada (*He*), número promedio de alelos por locus (A), índice de fijación de Wright ($F_{is}$) y diversidad genética entre poblaciones para diferentes especies de coníferas.

| Especies | N Pob | N loci | distribución | $H_e$ | A | $F_{is}$ | $G_{st}/F_{st}$ | Referencia |
|---|---|---|---|---|---|---|---|---|
| *A. balsamea* | | | | 0.274 | 2.05 | -0.008 | | Neale and Adams, 1985 |
| *A. balsamea* | 4 | 22 | 480 km | 0.018 | 1.21 | 0.154 | 0.037 | Shea and Furnier, 2002 |
| *A. fraseri* | | | | 0.286[b] | 1.10 | 0.007 | 0.002 | Diebel and Feret, 1991 |
| *A. alba* | | | | | | 0.006 | 0.071 | Breitenbach-Dorfer et al., 1997 |
| *A. alba* | | 17 | | 0.182 | 1.6 | | | Fady and Conkle, 1993 |
| *A. cephalonica* | | 17 | | 0.221 | 2.0 | 0.234 | 0.048 | Fady and Conkle, 1993 |
| *A. borisii regis* | | 14 | | 0.198 | 1.8 | | | Fady and Conkle, 1993 |
| *A. lasiocarpa* | | | | 0.124 | 1.6 | 0.341 | 0.017 | Shea, 1990 |
| *A. kawakamii* | | | | 0.283 | 2.2 | | | Kormutak and Yang, 1998 |
| *A. guatemalensis* | 10 | 16 | W | 0.069 | 1.38 | 0.235 | 0.122 | Este estudio |
| *A. hickeli* | 6 | 16 | N | 0.100 | 1.5 | 0.121 | 0.021 | Este estudio |
| *A. religiosa* | 11 | 16 | W | 0.108 | 1.5 | 0.216 | 0.250 | Este estudio |
| *A. flinckii* | 6 | 16 | N | 0.113 | 1.6 | 0.074 | 0.271 | Este estudio |
| *P. flexilis* | | | | 0.32 | 2.3 | | 0.022 | Schuster et al. 1989 |
| *P. flexilis* | 30 | | 1384.6 km | 0.166 | 2.46 | 0.108 | 0.101 | Jorgensen et al. 2002 |
| *P. balfouriana* | 16 | 11 | 766.7 km | 0.075 | 1.27 | 0.443south 0.203north | 0.075south 0.242north | Oline et al. 2000 |
| *P. longaeva* | 3 | 36 | 13.4 km | 0.122 | 1.92 | 0.078 | 0.011 | Lee et al. 2002 |
| *P. torreyana* | | | | | 2.2 | | 1.000 | Ledig and Conkle 1983 |
| *P. pinceana* | | | | 0.374 | 2.3 | 0.458 | 0.247 | Molina-Freaner et al. 2001 |
| *P. pinceana* | | | | 0.174 | 1.8 | 0.140 | 0.152 | Ledig et al. 2001 |
| *P. lagunae* | | | | 0.386 | 2.5 | 0.534 | 0.188 | Molina-Freaner et al. 2001 |
| *P. muricata* | | | | 0.346 | 2.1 | 0.307 | 0.161 | Molina-Freaner et al. 2001 |
| *P. engelmanni* | | | | 0.10 | 1.4 | | 0.130 | Bermejo 1993 |
| *P. rzedowskii* | | | | 0.22 | 1.8 | | 0.175 | Delgado et al. 1999 |
| *P. caribaea* | | | | 0.10 | 1.4 | | 0.130 | Matheson et al. 1989 |
| *P. oocarpa* | | | | 0.19 | 2.2 | | 0.104 | Matheson et al. 1989 |
| *Pseudotsuga menziesii* | 49 | 20 | 402.86 km | 0.163 | | 0.008 | 0.045 | El-Kassaby and Ritland 1990 |
| *Picea martinezii* | | | | 0.111 | 1.39 | 0.044 | 0.024 | Ledig et al. 2000 |

Tabla 2. Características morfológicas de *Abies guatemalensis* (Figura 2), *A. guatemalensis* var. *tacanensis* (considerada en este estudio como *A. guatemalensis*) *A. guatemalensis* var. *jaliscana*, (considerada en este estudio como *A. flinckii*) *A. religiosa* (Figura 3) *A. religiosa* var. *emarginata* (considerada en este estudio como *A. flinckii*), *A. hickeli* (Figura 4), *A. oaxacana* (considerada en este estudio como *A. hickeli*) y *A. flinckii* (Martínez 1948, Rushforth 1989, Farjon 1990, 1998)

| Especie | canales resiníferos | ramillas | hojas | ápice hojas | hoja largo | estomas | altura | diámetro | cono | bráctea | Notas |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. guatemalensis* | 2 sobre la epidermis o subepidermis, marginales | más o menos hirsutas, moradas rojizas | subdísticas o casi pectinadas extendidas casi en ángulo recto, acanaladas | obtuso o emarginado | 20-55mm | cara inferior de la hoja | 35m | 60-90cm | 8 a 11.5cm por 4 a 5.5 de ancho, subsésiles | inclusa | Especie reconocida por todos los autores |
| *A. guatemalensis* var. *tacanensis* considerado en este estudio como *A. guatemalensis* | 2 sobre la epidermis | muy hirsutas, rojizas | extendidas, acanaladas hasta los dos tercios a partir de la base en la cara superior | emarginado | 12-36mm | cara inferior rara vez en la superior | 35m | 60-90cm | 10cmm por 4 de ancho, subsésil | exerta o casi igualando la altura de la escama | Subespecie propuesta por Martínez (1948), pero actualmente reconocida por otros autores como *A. guatemalensis* |
| *A. religiosa* | 2 en la cara inferior, marginales, subepidermis | más o menos hirsutas, rojizas | subdísticas, alternas, en espirales, derechas o algo falcadas | agudo y córneo, casi erguidas | 20-30mm | cara superior e inferior de la hoja | 35-45m, hasta 50 o 60m | 1 a 1.50 o 1.80m | 10 a 16cm por 4 a 6 de ancho, casi sésiles o con pedúnculo de 5 a 9mm | exertas y reflejadas, sobresallendo de 8 a 10 mm | Especie reconocida por todos los autores |
| *A. hickeli* | 2 laterales en cara inferior, subepidermis y de 2 a 6 o hasta 10 accesorios centrales, subepidermis y tejido en palizada | hirsutas, moradas rojizas o rojizas cafés | subdísticas, pectinadas, lineares, derechas o ligeramente falcadas | angostado y claramente emarginado | 19-25mm. extendidas, algo pectinadas | cara inferior de la hoja | 20-30m | 70-90cm | 7 a 8cm de largo por 3.5 a 7 de ancho, subsésiles con pedunculo hasta de 10mm | exerta, muy salientes | Especie reconocida por todos los autores |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. oaxacana* considerado en este estudio como *A. hickeli* | 8-12 colocación variable, epidermis, subepidermis, clorénquima | Glabras con follaje aglomerado | Rectas, gruesas y tiesas | redondeado o truncado y ligeramente emarginado | 22-30mm | cara inferior de la hoja | 20-30m | 70-90cm | 9 a 12cm de largo por 4 a 5 de ancho, subsésiles o sésiles | poca saliente o igualando a la escama | Propuesta por Martinez (1948), pero considerada por otros autores como *A. hickeli* |
| *A. guatemalensis* var. *jaliscana* considerado en este estudio como *A. flinckii* | 2 en la cara inferior, cerca de las extremidades, subepidermis | hirsutas | subdísticas | emarginado o truncado | 26-65mm | cara superior e inferior de la hoja | 20-30m | 1.25m | 6 a 9cm por 2.5 a 3cm de anchocon pedúnculo corto de unos 10mm | inclusas llegando a los dos tercios de altura de la escama | Considerada como *A. flinckii* por Rushforth (1989) |
| *A. religiosa* var. *emarginata* considerado en este estudio como *A. flinckii* | 2 en la subepidermis | hirsutas | subdísticas, lineares, torcidas en la base | emarginado o truncado | 25-50mm, a veces hasta 60 o 70 mm | cara superior e inferior de la hoja | 45m | 60cm a 1.20m | 12.5 a 13.5 cm de largo por 3 a 4 de ancho | exerta, salientes y reflejadas | Considerada como *A. flinckii* por Rushforth (1989) |
| *A. flinckii* | 2 | | dísticas o subdísticas | hendido | | | | | | exerta/ inclusa | Rushforth (1989) Agrupa a *A. guatemalensis* var. *jaliscana* y a *A. religiosa* var. *emarginata* |

Tabla 3. Coordenadas geográficas y altitud (m) de las poblaciones de *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H) y *A. religiosa* (R) estudiadas.

| Población Municipio y estado | Especie | Lat N/ long W | altitud |
|---|---|---|---|
| **F14** Hidalgo, Michoacán | *A. flinckii* (Rushforth 1989) *A. religiosa* var. *emarginata* (Martínez 1948) | 19° 35' 100° 45' | 2340 |
| **F16** Villa Madero, Michoacán | *A. flinckii* (Rushforth 1989) *A. religiosa* var. *emarginata* (Martínez 1948) | 19° 20' 101° 21' | 2250 |
| **F17** Aguililla, Michoacán | *A. flinckii* (Rushforth 1989) *A. religiosa* var. *emarginata* (Martínez 1948) | 18° 46' 102° 57' | 2500 |
| **F18** Talpa de Allende, Jalisco | *A. flinckii* (Rushforth 1989) *A. guatemalensis* var. *jaliscana* (Martínez 1948) | 20° 12' 104° 43' | 2100 |
| **F19** Talpa de Allende, Jalisco | *A. flinckii* (Rushforth 1989) *A. guatemalensis* var. *jaliscana* (Martínez 1948) | 20° 21' 104° 59' | 2490 |
| **F20** Minantitlán, Colima | *A. flinckii* (Rushforth 1989) *A. religiosa* var. *emarginata* (Martínez 1948) | 19° 27' 103° 56' | 2500 |
| **G2** Santa Catarina Ixtepeji, Oaxaca | *A. guatemalensis* (Martínez 1948) | 17° 10' 96° 30' | 2800 |
| **G7** San Sebastián Río Hondo, Oaxaca | *A. guatemalensis* (Martínez 1948) | 16° 11' 96° 18' | 2500 |
| **G10** Cacahoatán, Chiapas | *A. guatemalensis* (Martínez 1948) *A. guatemalensis* var. *tacanensis* (Martínez 1948) | 15° 07' 92° 07' | 3330 |
| **G11** El Porvenir, Chiapas | *A. guatemalensis* (Martínez 1948) | 15° 27' 92° 16' | 2610 |
| **G41** El Progreso, Guatemala | *A. guatemalensis* (Martínez 1948) | 15° 04' 89° 55' | 2970 |
| **G42** Jalapa, Guatemala | *A. guatemalensis* (Martínez 1948) | 14° 31' 90° 08' | 2610 |

| | | | |
|---|---|---|---|
| **G43**<br>Sololá,<br>Guatemala | *A. guatemalensis* (Martínez 1948) | 14° 52'<br>91° 17' | 2730 |
| **G44**<br>Leonardo Bravo,<br>Guerrero | *A. guatemalensis* (Martínez 1948) | 17° 35'<br>99° 51' | 2670 |
| **G51**<br>Ciudad del Maíz,<br>San Luis Potosí | *A. guatemalensis* (Martínez 1948) | 22° 27'<br>99° 27' | 1770 |
| **G52**<br>Agua Blanca de<br>Iturbide, Hidalgo | *A. guatemalensis* (Martínez 1948) | 20° 21'<br>98° 20' | 2310 |
| **H1**<br>Santa Catarina<br>Lachatao,<br>Oaxaca | *A. hickeli* (Martínez 1948)<br>*A. oaxacana* (Martínez 1948) | 17° 10'<br>96° 22' | |
| **H3**<br>Ixtlán de Juarez,<br>Oaxaca | *A. hickeli* (Martínez 1948)<br>*A. oaxacana* (Martínez 1948) | 17° 22'<br>96° 26' | 2904 |
| **H4**<br>Ixtlán de Juarez,<br>Oaxaca | *A. nickeli* (Martínez 1948)<br>*A. zapotekensis* (Debreczy and Racz 1995) | 17° 27'<br>96° 24' | 2530 |
| **H5**<br>Zimatlán de<br>Alvarez, Oaxaca | *A. hickeli* (Martínez 1948) | 16° 44'<br>97° 07' | 2600 |
| **H9**<br>Santa María<br>Yucuhiti, Oaxaca | *A. hickeli* (Martínez 1948) | 17° 03'<br>97° 45' | 3000 |
| **H46**<br>Xometla,<br>Veracruz | *A. hickeli* (Martínez 1948) | 18° 58'<br>97°12' | 2910 |
| **H47**<br>Peróte, Veracruz | *A. hickeli* (Martínez 1948) | 19° 31'<br>97°06' | 3090 |
| **R12**<br>Zinacantepec,<br>Edo. de México | *A. religiosa* (Martínez 1948) | 19° 11'<br>99° 48' | 3240 |
| **R13**<br>Villa de Allende<br>Edo. de México | *A. religiosa* (Martínez 1948) | 19° 26'<br>100° 10' | 2800 |
| **R15**<br>Querándaro,<br>Michoacán | *A. religiosa* (Martínez 1948) | 19° 40'<br>100° 49' | 2880 |

| | | | |
|---|---|---|---|
| **R21**<br>Minantitlán, Colima | *A. religiosa* (Martínez 1948) | 19° 27'<br>103° 56' | 2500 |
| **R22**<br>Zapotitlán, Jalisco | *A. religiosa* (Martínez 1948)<br>*A. colimensis* (Rushforth 1989) | 19° 35'<br>103° 35' | 3330 |
| **R45**<br>Ciudad Serdán, Puebla | *A. religiosa* (Martínez 1948) | 18° 58'<br>97° 21' | 3060 |
| **R48**<br>Peróte, Veracruz | *A. religiosa* (Martínez 1948) | 19° 31'<br>97° 09' | 3510 |
| **R49**<br>Tlaxco, Tlaxcala | *A. religiosa* (Martínez 1948) | 19° 41'<br>98° 05' | 2760 |
| **R53**<br>Mineral Real del Monte, Hidalgo | *A. religiosa* (Martínez 1948) | 20° 09'<br>98° 42' | 2940 |
| **R54**<br>Atlautla, Edo. de México | *A. religiosa* (Martínez 1948) | 19° 23'<br>98° 40' | 3330 |
| **R55**<br>Tancítaro, Michoacán | *A. religiosa* (Martínez 1948) | 19° 23'<br>102° 19' | 3030 |

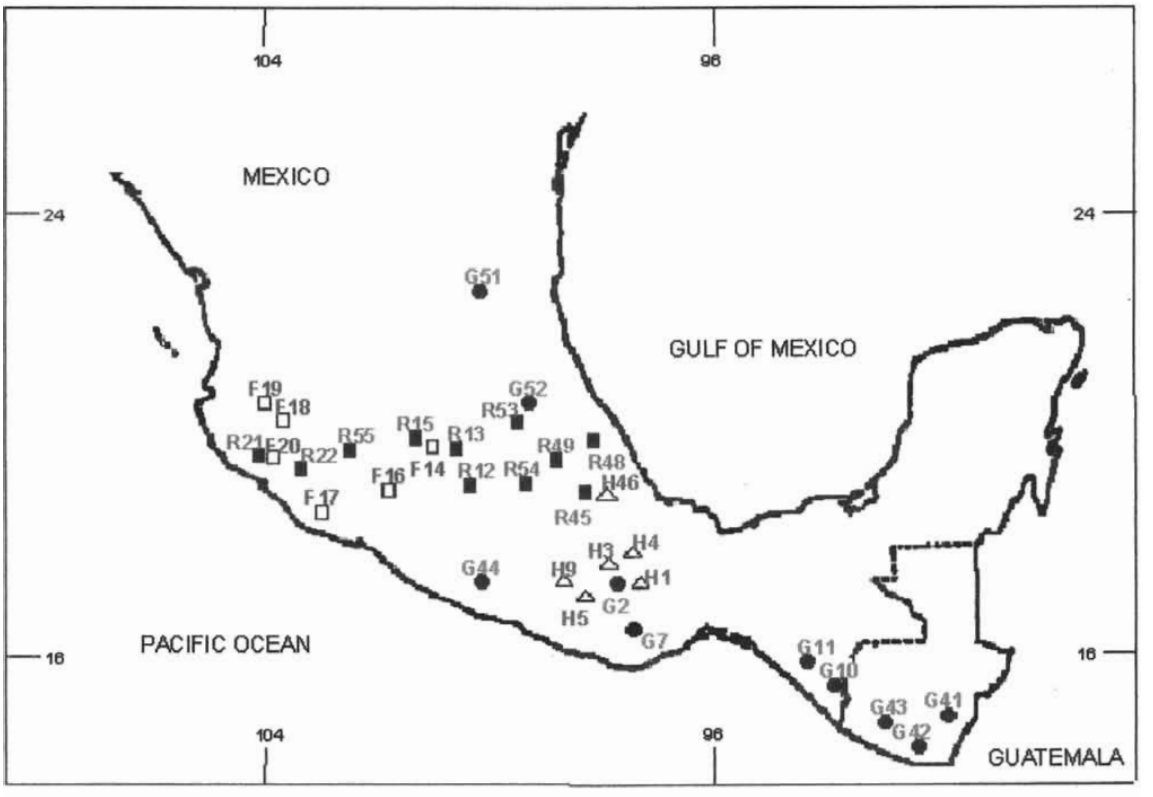


Figura 1. Distribución de las poblaciones estudiadas de A. *guatemalensis* (G), A. *religiosa* (R), *A. hickeli* (H) y *A. Flinckii* (F). Información sobre coordenadas geográficas y altitud de cada población en la Tabla 3.

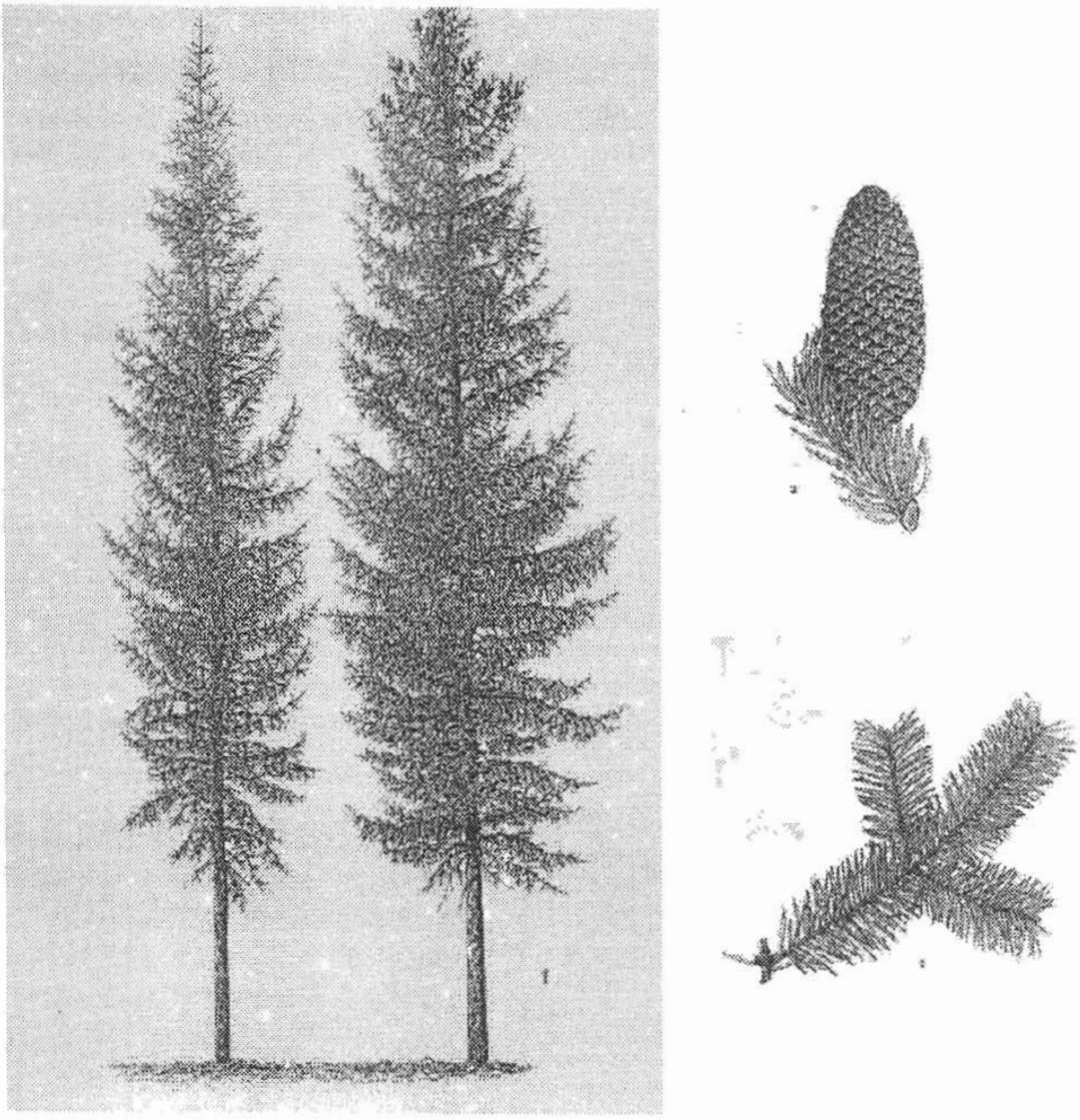

Figura 2. Dibujo de las características de la hoja y el cono de *Abies guatemalensis* (Farjon 1990)

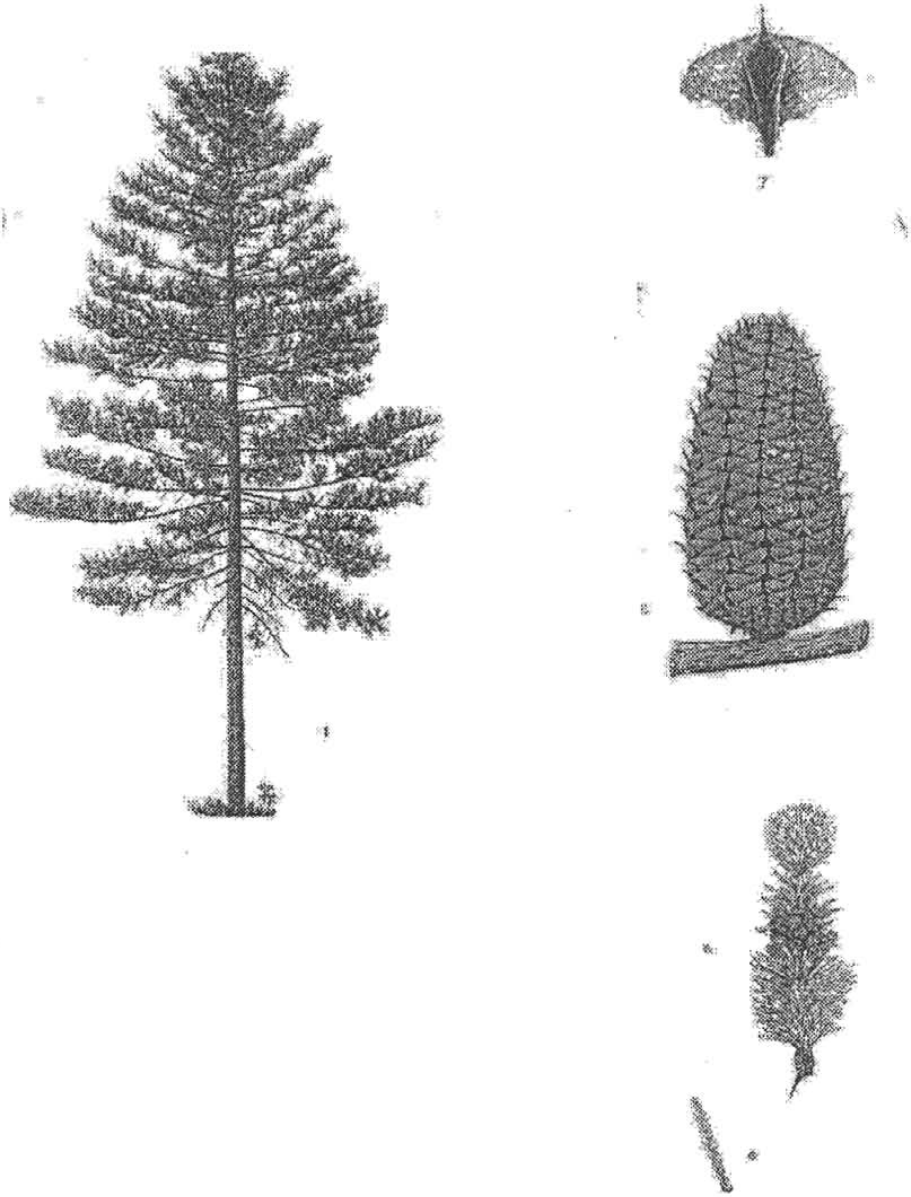

Figura 3. Dibujo de las características de la hoja, cono y escama de *Abies religiosa* (Farjon 1990)

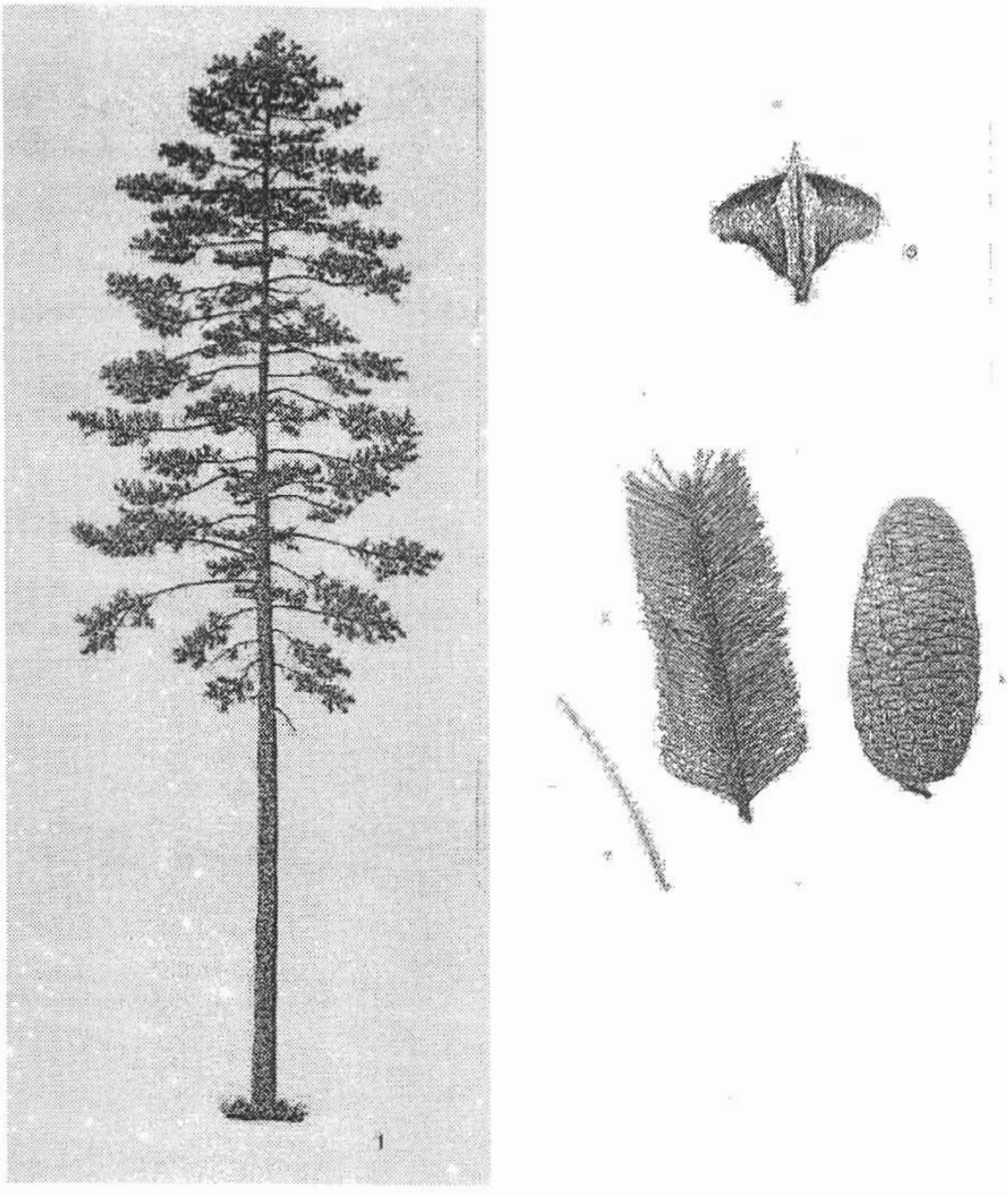

Figura 4. Dibujo de las características de la hoja, cono y escama de *Abies hickeli* (Farjon 1990)

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# LOW LEVELS OF GENETIC VARIATION WITHIN AND HIGH LEVELS OF GENETIC DIFFERENTIATION AMONG POPULATIONS OF SPECIES OF *ABIES* FROM SOUTHERN MEXICO AND GUATEMALA[1]

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Populations of *Abies* in southern Mexico and Guatemala (*A. flinckii, A. guatemalensis, A. hickeli,* and *A. religiosa*) have a patchy distribution. This pattern is particularly clear in *A. guatemalensis.* Genetic diversity within populations, measured by average heterozygosity at 16 isozyme loci, is lower than the range reported for most conifers (mean $H_o$ ranging from 0.069 in *A. guatemalensis* to 0.113 in *A. flinckii*), while differentiation among populations is higher than that observed in most conifer species studied ($\theta = F_{st}$ ranging from 0.073 in *A. hickeli* to 0.271 in *A. flinckii*). Estimated levels of gene flow are low (ranging from 0.672 in *A. flinckii* to 3.17 in *A. hickeli*). Populations in most cases had an excess of homozygosity over that expected under Hardy-Weinberg equilibrium, suggesting some inbreeding ($F_{is}$ ranging from 0.074 in *A. flinckii* to 0.235 in *A. guatemalensis*). A significant relationship between gene flow and geographic distance was observed in *A. religiosa,* but not in the other three taxa studied. The patterns of genetic variation appear to have been influenced by the distributions and histories of these species. Paleoclimatic evidence suggests that the ranges of these species retreated upwards during the Pleistocene glaciation and became fragmented during the warming period that followed. The populations could have passed through genetic bottlenecks that reduced genetic variation and led to interpopulation differentiation.

Key words: *Abies flinckii*; *Abies guatemalensis*; *Abies hickeli*; *Abies religiosa*; gene flow; genetic variation; genetic drift; inbreeding.

Most conifers studied have high levels of genetic diversity, as measured by isozymes (Hamrick, Godt, and Sherman-Broyles, 1992; Fady and Conkle, 1993; Matusova, 1995; El-Kassaby and Ritland, 1996). It has also been found that the majority of forest tree species show low levels of isozyme differentiation among populations. These studies have mostly been conducted with economically important conifers of widespread and often continuous distributions. The high within-population genetic diversity and low among-population differentiation observed in conifers have been attributed to common life-history traits, such as longevity and extensive gene flow (Hamrick, Godt, and Sherman-Broyles, 1992; Streiff et al., 1998). However, the biogeographic history of a species should also contribute significantly to current patterns of genetic variation.

The *Abies* species of southern Mexico and Guatemala provide an excellent opportunity to study the effects of species distributions on patterns of genetic variation because the different species have contrasting geographical distributions. There are ~50 species of *Abies* (firs) in the world, distributed in the temperate regions of the Northern Hemisphere (Welch, 1991). Of these, Martínez (1948) listed eight species as occurring in Mexico, six of them endemic to the country. Liu (1971) reduced this number to six species, four of them endemic to Mexico. Farjon (1990) listed six species in Mexico, four of them endemic to the country. Rushforth (1989) has since described two additional species from western Mexico, and Debreczy and Racz (1995) have described three more.

We considered four species of *Abies* that occur in southern Mexico and Guatemala. *Abies guatemalensis* Rehder is the southernmost representative of the genus, distributed at altitudes between 2000 and 4000 m in El Salvador, Guatemala, Honduras, and the southern Mexican states of Chiapas, Oaxaca, Guerrero, Jalisco, Hidalgo, and San Luis Potosí (Martínez, 1948; Donahue et al., 1985). Although widely distributed, it has relatively few isolated populations and is considered a threatened species (FAO, 1986). *Abies religiosa* (H. B. K.) Schl. et Cham. is distributed principally above 2000 m along the Transversal Volcanic Belt, in the states of Guerrero, Jalisco, Michoacán, México, Morelos, Hidalgo, Tlaxcala, Puebla, and Veracruz and the Distrito Federal (Martínez, 1948). Its distribution is more continuous than that of *A. guatemalensis. Abies religiosa* var. *emarginata* Martínez is distributed in the states of Jalisco and Michoacán, and *A. guatemalensis* var. *jaliscana* Martínez has a very re-

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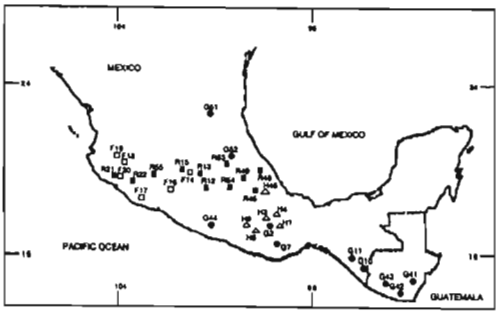


Fig. 1. Locations of populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R) sampled for isozyme analysis.

stricted distribution in western Jalisco. Martínez (1948) described these two varieties, but Rushforth (1989) subsequently combined them to form a new species, *A. flinckii* Rushforth. Our field observations suggest that these populations are indeed morphologically similar to each other and distinct from *A. religiosa* and *A. guatemalensis*. They differ even more strikingly in phenology from the latter two species, beginning vegetative growth as much as 3 mo earlier. The maintenance of very different morphologies and phenologies in trees of *A. flinckii* and *A. religiosa* intermixed at the same site in the Sierra de Manantlán strongly suggests that these are reproductively isolated separate species. *Abies hickeli* Flous et Gaussen has a limited range, with a few populations in the states of Oaxaca and Veracruz (Martínez, 1948).

In this study, we used isozyme markers to examine the levels and patterns of genetic variation in the *Abies* of southern Mexico and Guatemala. In particular, we were interested in the effects of species distribution and historical factors, such as habitat fragmentation and isolation of populations due to climatic changes.

## MATERIALS AND METHODS

Branch samples were collected from 33 populations of *Abies* in southern Mexico and Guatemala. Included were ten populations of *A. guatemalensis*, 11 of *A. religiosa*, six of *A. flinckii*, and six of *A. hickeli* (Fig. 1). These populations represent most of the geographic range covered by these species. Because of our need to collect vegetative buds, the short period in which *Abies* cones are intact before disintegrating, and the large geographic area we sampled, we were unable to sample cones from these populations in the field. The populations we sampled were, however, previously described based on vegetative and reproductive characteristics by Martínez (1948), Donahue et al. (1985), and/or samples in the Herbario Nacional (MEXU). Forty individuals were sampled in each population, when possible. In the laboratory, vegetative buds were removed from the branch samples and were stored at −80°C for further use in gel electrophoresis. We maintained branch samples from each population as vouchers.

Standard methods for gel electrophoresis were followed (Conkle et al., 1982; Cheliak and Pitel, 1984). Buds from each individual were ground with extraction buffer (3:1 [v:v] mixture of buffer YO from Yeh and O'Malley [1980] and Vegll from Pitel and Cheliak [1984]). The extract was absorbed in 1.2 × 1.5 mm chromatographic paper wicks. The enzyme analysis was done using two systems of electrode and gel buffers. The H buffer (Cheliak and Pitel, 1984) was used to assay shikimate dehydrogenase (SDH, Enzyme Commission number 1.1.1.25, two loci), isocitrate dehydrogenase (IDH, E.C. 1.1.1.41, one locus), phosphoglucomutase (PGM, E.C. 2.7.5.1, two loci), and menadione reductase (MNR, E.C. 1.6.99.2, one locus). The R buffer (Ridgeway, Sherburne, and Lewis, 1970) was used to assay leucine amino-peptidase (LAP, E.C. 3.4.11.1, one locus), phosphoglucoisomerase (PGI, E.C. 5.3.1.9, two loci), glutamic-oxaloacetic transaminase (GOT, E.C. 2.6.1.1, one locus), peroxidase (PER, E.C. 1.11.1.7, one locus), peptidase (PEP, E.C. 3.4.11.11, three loci), glutamate dehydrogenase (GDH, E.C. 1.4.1.3, one locus), and ribulosebiphosphate carboxylase (RUB, E.C. 4.1.1.39, one locus). Starch gels (12%) were run at 60 mA for 6–7 h on the H system and 4 h on the R system. All genotypic data for all individuals in this study are in the databases of the Comisión para el Uso y Conocimiento de la Biodiversidad (CONABIO; http://www.conabio.gob.mx) in Mexico City.

Allelic frequencies were obtained for each locus in each population. Based on these data, levels of polymorphism (P), observed ($H_o$) and Hardy-Weinberg expected ($H_e$) heterozygosity, and mean number of alleles per locus ($A$) were estimated with the program BIOSYS (Swofford and Selander, 1981). Wright's (1965) $F$ statistics, total inbreeding ($F_{is}$), subdivision among populations ($F_{st}$), and inbreeding within populations ($F_{is}$) were estimated for each species by the method described in Weir and Cockerham (1984) ($F = F_{is}$, $\theta = F_{st}$ and $f = F_{is}$), using the program FSTAT (version 1.2; Goudet, 1995). Partitioning of variation within and among species was estimated using the WRIGHT78 procedure of the program BIOSYS (Swofford and Selander, 1981).

An estimate of gene flow ($Nm$), the average effective number of migrants exchanged between populations in each generation, was calculated from $\theta$, and also from the number and frequency of private alleles (Barton and Slatkin, 1986). Wright (1951) showed that in an island model, where equilibrium has been reached between genetic drift and migration, $Nm = (1 - \theta)/4\theta$, where $\theta$ is the proportion of total genetic diversity represented by differences among populations.

$Nm$ can also be calculated from the number and frequency of private alleles, unique alleles found in only one population (Slatkin, 1985), as follows:

$$\log_{10}[p(1)] = a \log_{10}(Nm) + b,$$

where $p(1)$ is the mean frequency of private alleles and $a$ and $b$ are constants determined by simulated data. We used values for $a$ and $b$ obtained for a sample size of 25 and corrected for our mean sample size (Barton and Slatkin, 1986). This method of estimating gene flow is likely to be more sensitive to errors in data collection (Slatkin, 1994).

The relationship of gene flow between pairs of populations ($M$) to geographic distance (Slatkin, 1993) was examined. Values of $M$ were obtained by computing $\theta$ (a measure of among-population differentiation; Weir and Cockerham, 1984) for each pair of locations using a program provided by Slatkin (isolation by distance program). A regression of $\log_{10}M$ on $\log_{10}k$ ($k$ = geographic distance) was then constructed to see if there was a linear relationship. The significance of the relationship was evaluated by Mantel's (1967) test, since the usual statistical methods cannot be used because values of $M$ from different pairs of populations are not independent. This was done for each taxon studied. Finally, we used PHYLIP (Felsenstein, 1995) to construct a neighbor joining tree (Saitou and Nei, 1987), based on Nei's unbiased genetic distances (Nei, 1978). One hundred bootstrap samples over loci were used to estimate the confidence of the branch points.

## RESULTS

Levels of polymorphism and expected average heterozygosity were lowest for *A. guatemalensis*, intermediate for *A. hickeli*, and highest for *A. flinckii* and *A. religiosa* (Table 1). The range of variation among populations in

TABLE 1. Geographic coordinates, elevation (m), mean number of individuals analyzed per locus ($N$), mean number of alleles per locus ($A$), percentage of polymorphic loci (frequency of most common allele < 0.95) (P), observed heterozygosity ($H_o$), mean unbiased estimate of expected heterozygosity ($H_e$), and fixation index ($F$) across 16 isozyme loci for populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R). Standard errors are in parentheses.

| Population code | Lat N/ long W | elevation | $N$ | $A$ (SE) | P(%) | $H_o$ (SE) | $H_e$ (SE) | $F$ (SE) |
|---|---|---|---|---|---|---|---|---|
| F14 | 19°35′ 100°45′ | 2340 | 26.6 | 1.7 (0.2) | 25.0 | 0.055 (0.024) | 0.089 (0.035) | 0.284 (0.444) |
| F16 | 19°20′ 101°21′ | 2250 | 24.4 | 1.6 (0.2) | 37.5 | 0.184 (0.059) | 0.158 (0.049) | −0.120 (0.271) |
| F17 | 18°46′ 102°57′ | 2500 | 19.6 | 1.4 (0.2) | 12.5 | 0.031 (0.018) | 0.064 (0.037) | 0.322 (0.550) |
| F18 | 20°12′ 104°43′ | 2100 | 39.3 | 1.7 (0.2) | 37.5 | 0.125 (0.043) | 0.125 (0.044) | −0.006 (0.253) |
| F19 | 20°21′ 104°59′ | 2490 | 34.6 | 1.8 (0.2) | 37.5 | 0.119 (0.048) | 0.130 (0.044) | 0.165 (0.426) |
| F20 | 19°27′ 103°56′ | 2500 | 29.9 | 1.4 (0.2) | 31.3 | 0.097 (0.041) | 0.109 (0.047) | 0.027 (0.205) |
| *A. flinckii* mean | | | 29.1 | 1.6 (0.17) | 30.2 (10.0) | 0.102 (0.054) | 0.113 (0.033) | 0.112 (0.174) |
| G2 | 17°10′ 96°36′ | 2800 | 30.8 | 1.3 (0.1) | 25.0 | 0.047 (0.022) | 0.060 (0.027) | 0.124 (0.378) |
| G7 | 16°11′ 96°18′ | 2500 | 25.4 | 1.3 (0.1) | 18.8 | 0.033 (0.016) | 0.044 (0.023) | 0.139 (0.254) |
| G10 | 15°07′ 92°07′ | 3330 | 28.8 | 1.4 (0.1) | 25.0 | 0.051 (0.028) | 0.072 (0.032) | 0.250 (0.456) |
| G11 | 15°27′ 92°16′ | 2610 | 36.1 | 1.4 (0.2) | 18.8 | 0.072 (0.032) | 0.070 (0.033) | 0.107 (0.440) |
| G41 | 15°04′ 89°55′ | 2970 | 35.8 | 1.3 (0.1) | 18.8 | 0.043 (0.020) | 0.058 (0.027) | 0.173 (0.280) |
| G42 | 14°31′ 90°08′ | 2610 | 20.5 | 1.3 (0.1) | 12.5 | 0.046 (0.027) | 0.058 (0.036) | 0.083 (0.139) |
| G43 | 14°52′ 91°17′ | 2730 | 25.7 | 1.3 (0.1) | 12.5 | 0.047 (0.025) | 0.063 (0.036) | 0.079 (0.244) |
| G44 | 17°35′ 99°51′ | 2670 | 32.8 | 1.4 (0.1) | 18.8 | 0.044 (0.024) | 0.079 (0.041) | 0.160 (0.387) |
| G51 | 22°27′ 99°27′ | 1770 | 27.6 | 1.4 (0.2) | 25.0 | 0.075 (0.038) | 0.090 (0.043) | 0.209 (0.285) |
| G52 | 20°21′ 98°20′ | 2310 | 33.3 | 1.7 (0.2) | 25.0 | 0.070 (0.031) | 0.094 (0.036) | 0.331 (0.478) |
| *A. guatemalensis* mean | | | 29.7 | 1.38 (0.123) | 20.0 (4.9) | 0.053 (0.014) | 0.069 (0.015) | 0.165 (0.079) |
| H1 | 17°10′ 96°22′ | | 30.8 | 1.6 (0.2) | 31.3 | 0.041 (0.017) | 0.090 (0.034) | 0.394 (0.451) |
| H3 | 17°22′ 96°26′ | 2904 | 31.4 | 1.4 (0.2) | 18.8 | 0.055 (0.023) | 0.087 (0.039) | 0.179 (0.277) |
| H4 | 17°27′ 96°24′ | 2530 | 10.6 | 1.3 (0.1) | 12.5 | 0.078 (0.055) | 0.073 (0.047) | −0.125 (0.551) |
| H5 | 16°44′ 97°07′ | 2600 | 38.9 | 1.6 (0.2) | 43.8 | 0.145 (0.056) | 0.135 (0.051) | −0.002 (0.270) |
| H9 | 17°03′ 97°45′ | 3000 | 39.8 | 1.6 (0.2) | 18.8 | 0.091 (0.045) | 0.087 (0.042) | −0.047 (0.062) |
| H46 | 18°58′ 97°12′ | 2910 | 28.6 | 1.7 (0.2) | 43.8 | 0.115 (0.042) | 0.125 (0.045) | 0.108 (0.357) |
| *A. hickeli* mean | | | 30.2 | 1.53 (0.15) | 28.2 (13.6) | 0.088 (0.038) | 0.100 (0.025) | 0.085 (0.186) |
| R12 | 19°11′ 99°48′ | 3240 | 31.1 | 1.8 (0.2) | 43.8 | 0.121 (0.039) | 0.155 (0.048) | 0.133 (0.275) |
| R13 | 19°26′ 100°10′ | 2800 | 29.1 | 1.4 (0.2) | 31.3 | 0.070 (0.031) | 0.087 (0.039) | 0.104 (0.207) |
| R15 | 19°40′ 100°49′ | 2880 | 35.9 | 1.4 (0.1) | 31.3 | 0.051 (0.031) | 0.080 (0.036) | 0.332 (0.512) |
| R21 | 19°27′ 103°56′ | 2500 | 36.3 | 1.1 (0.1) | 0.0 | 0.009 (0.006) | 0.008 (0.006) | −0.036 (0.006) |
| R22 | 19°35′ 103°35′ | 3330 | 31.6 | 1.1 (0.1) | 12.5 | 0.033 (0.023) | 0.032 (0.024) | −0.041 (0.089) |
| R45 | 18°58′ 97°21′ | 3060 | 36.5 | 1.8 (0.2) | 37.5 | 0.105 (0.038) | 0.128 (0.041) | 0.136 (0.320) |

TABLE 1. Continued.

| Population code | Lat N/ long W | elevation | N | A (SE) | P(%) | $H_o$ (SE) | $H_e$ (SE) | F (SE) |
|---|---|---|---|---|---|---|---|---|
| R48 | 19°31′ | 3510 | 37.9 | 1.6 | 37.5 | 0.116 | 0.118 | −0.032 |
| | 97°09′ | | | (0.2) | | (0.037) | (0.039) | (0.140) |
| R49 | 19°41′ | 2760 | 34.7 | 1.6 | 25.0 | 0.099 | 0.116 | 0.183 |
| | 98°05′ | | | (0.2) | | (0.044) | (0.050) | (0.247) |
| R53 | 20°09′ | 2940 | 38.1 | 1.4 | 25.0 | 0.065 | 0.088 | 0.143 |
| | 98°42′ | | | (0.2) | | (0.032) | (0.042) | (0.262) |
| R54 | 19°23′ | 3330 | 26.6 | 2.2 | 68.8 | 0.132 | 0.235 | 0.398 |
| | 98°40′ | | | (0.2) | | (0.033) | (0.048) | (0.301) |
| R55 | 19°23′ | 3030 | 25.6 | 1.6 | 37.5 | 0.099 | 0.139 | 0.283 |
| | 102°19′ | | | (0.1) | | (0.047) | (0.049) | (0.503) |
| *A. religiosa* mean | | | 33.0 | 1.54 | 31.8 | 0.082 | 0.108 | 0.146 |
| | | | | (0.32) | (17.6) | (0.039) | (0.061) | (0.148) |

expected heterozygosity was particularly high in *A. religiosa,* which included the population with the lowest genetic diversity (R21, $H_e$ = 0.008), as well as the population with the highest value (R54, $H_e$ = 0.235).

In most populations the fixation index ($F$) was near zero or positive (Table 1). The estimates were high in all species, with *A. hickeli* having the lowest value. Standard errors of $F$ estimates for the populations were in general very high. The mean coefficient of inbreeding within subpopulations ($f = F_{is}$) and the total inbreeding coefficient ($F = F_{it}$) estimates are generally positive, with the highest average values for *A. guatemalensis* and *A. religiosa* and the lowest for *A. flinckii* (Table 2). There is significant differentiation among populations in all four species, with relatively high $\theta = F_{st}$ estimates for all species, particularly *A. flinckii* and *A. religiosa* (Table 2). The mean estimate of differentiation among populations ($F_{st} = \theta$) considering all species is 0.280, with 0.221 due to differences among populations within species and only 0.056 due to differences among species.

The mean estimates of *Nm* obtained from θ for each species were 0.672 (95% confidence interval = 0.46–1.43) for *A. flinckii,* 1.8 (1.34–3.72) for *A. guatemalensis,* 0.75 (0.43–1.57) for *A. religiosa,* and 3.17 (1.83–11.65) for *A. hickeli,* with an overall mean of 1.6 (obtained with the mean θ, when we analyze all species together). Estimates of *Nm* calculated from the method of private alleles were 3.42 for *A. flinckii,* 2.88 for *A. guatemalensis,* 1.67 for *A. religiosa,* and 2.70 for *A. hickeli.* Estimates of *Nm* from private alleles were higher than estimates from θ. It has been shown that both methods can provide accurate estimates of *Nm* under several circumstances (Slatkin and Barton, 1989), but the method of private alleles is more sensitive to errors in data collection and could be less accurate in practice (Slatkin, 1994). Simulations done by Slatkin (1993) show that in a stepping stone model, $M$ (gene flow) and geographic distance should be inversely correlated, with a slope of −1 in linearly distributed populations. This pattern was observed in *A. religiosa,* but not in any of the other species (Fig. 2).

The mean genetic distance (Nei, 1978) between populations of *A. flinckii* was 0.051 (SD = 0.035), between populations of *A. guatemalensis* 0.011 (0.009), between populations of *A. religiosa* 0.040 (0.033), and between populations of *A. hickeli* 0.010 (0.006). These estimates of genetic distances, particularly for *A. religiosa* and *A. flinckii,* are relatively high compared to other north-temperate conifers and show high differentiation between populations. This is in agreement with the estimates of θ. Considering all populations of all species together, the mean overall genetic distance was 0.040, similar to the mean genetic distances between populations within the same species. The phenogram constructed from these distances showed a monophyletic group for *A. flinckii,* within a group containing eight of the ten *A. guatemalensis* populations and distinct from the *A. religiosa* populations (Fig. 3). *Abies hickeli* populations from Oaxaca also formed a monophyletic group. *Abies religiosa* populations formed another monophyletic group that also included two populations of *A. guatemalensis* and the one population of *A. hickeli* from Veracruz. Within this group, there were two subgroups, one consisting of populations from the Mexico City area and west (G44, R12, R13, R15, R21, R22, R49, and R53) and the other consisting of populations principally from the Mexico City area and east (G52, H46, R45, R48, and R54, with R55 being the exception). Levels of confidence in the branch points of this phenogram are relatively low.

## DISCUSSION

The levels of genetic diversity we observed in these *Abies* species were lower than means reported from studies of other gymnosperms (P = 71.1, $A$ = 1.83, $H_e$ = 0.151), other temperate zone species (P = 63.5, $A$ = 1.81, $H_e$ = 0.166), and other species with outcrossing mating systems and wind-dispersed pollen (P = 53.0, $A$ = 1.84, $H_e$ = 0.173) (Hamrick, Godt, and Sherman-Broyles, 1992). The estimates were also lower than those reported for other species of *Abies* (Table 3). There are relatively few reports of natural populations of tree species with very low levels of genetic variation. Such low levels of variation usually occur in species with very narrow geographic distributions, species that are inbreeding colonizers (Brown and Marshall, 1981), or species with very small population sizes (Hartl and Clark, 1989). In many populations we observed hundreds of individuals, enough to counteract the effects of genetic drift. In some of the populations, however, we observed relatively low total sizes, particularly for *A. guatemalensis,* a species in which we found lower levels of heterozygosity. Estimates of expected heterozygosity varied from almost twofold to almost 30-fold among populations within species (0.064–

TABLE 2. Weir and Cockerham's (1984) estimates of Wright's $F$ statistics calculated for each locus for all populations of *Abies flinckii, A. guatemalensis, A. hickeli,* and *A. religiosa.* Significance of deviations from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$) were tested by using permutations. Means and standard deviations were obtained by jackknifing over loci, and confidence intervals were obtained by bootstrapping over loci.

| | *A. flinckii* | | | *A. guatemalensis* | | | *A. hickeli* | | | *A. religiosa* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | $F_{it}$ | $F_{st}$ | $\theta$ | $F_{it}$ | $F_{st}$ | $\theta$ | $F_{it}$ | $F_{st}$ | $\theta$ | $F_{it}$ | $F_{st}$ | $\theta$ |
| *Gdh1* | 1.000*** | 1.000*** | −0.004 | 1.000*** | 1.000*** | 0.003 | 1.000*** | 1.000*** | 0.043*** | 0.866*** | 0.878*** | 0.090*** |
| *Got1* | 0.129 | 0.238*** | 0.125*** | 0.687*** | 0.754*** | 0.213*** | 0.120* | 0.216*** | 0.109*** | 0.401*** | 0.590*** | 0.316*** |
| *Idh2* | −0.187 | −0.125 | 0.052*** | −0.138 | −0.009 | 0.114*** | −0.215* | 0.010 | 0.185*** | 0.253*** | 0.288*** | 0.048*** |
| *Lap1* | 1.000*** | 1.000*** | 0.053*** | 0.722*** | 0.748*** | 0.093 | −0.032 | −0.008 | 0.023* | 0.110 | 0.200*** | 0.101*** |
| *Mnr1* | 1.000*** | 1.000*** | 0.011 | | | | 0.002 | −0.001 | −0.003 | | | |
| *Pep1* | | | | | | | | | | 0.660*** | 0.667*** | 0.020** |
| *Pep3* | | | | | | | | | | 0.498*** | 0.498*** | 0.001 |
| *Per3* | 0.067 | 0.310*** | 0.260*** | 0.058 | 0.142*** | 0.089*** | 0.005 | 0.023 | 0.018 | −0.021 | 0.141* | 0.159*** |
| *Pgi1* | 0.125 | 0.160** | 0.040*** | 0.321* | 0.373*** | 0.076*** | −0.022 | −0.019 | 0.003 | 0.171*** | 0.317*** | 0.176*** |
| *Pgi2* | 0.365*** | 0.586*** | 0.348*** | −0.024 | −0.002 | 0.021* | 0.325*** | 0.325*** | 0.004 | 0.810*** | 0.967*** | 0.824*** |
| *Pgm1* | 0.025 | 0.465*** | 0.451*** | 0.035 | 0.099 | 0.035*** | 0.345*** | 0.362** | 0.026** | 0.258*** | 0.303*** | 0.060*** |
| *Pgm2* | −0.118 | 0.116 | 0.209*** | −0.066 | −0.040 | 0.024** | −0.068 | −0.020 | 0.045* | 0.242*** | 0.340*** | 0.128*** |
| *Sdh1* | −0.153 | −0.006 | 0.128*** | 0.195*** | 0.303*** | 0.134*** | 0.200*** | 0.217*** | 0.021 | 0.100* | 0.302*** | 0.225*** |
| *Sdh2* | 0.025 | 0.436*** | 0.421*** | 1.000*** | 1.000*** | 0.095*** | | | | 0.219* | 0.340*** | 0.155*** |
| Mean | 0.074** | 0.325*** | 0.271*** | 0.235*** | 0.330*** | 0.122*** | 0.121*** | 0.183*** | 0.073*** | 0.216*** | 0.414*** | 0.250*** |
| SD | (0.057) | (0.072) | (0.058) | (0.101) | (0.106) | (0.024) | (0.065) | (0.046) | (0.030) | (0.078) | (0.089) | (0.054) |
| 95% CI | −.027–0.194 | 0.166–0.438 | 0.149–0.351 | 0.080–0.477 | 0.144–0.557 | 0.063–0.157 | −0.025–0.243 | 0.079–0.275 | 0.021–0.120 | 0.106–0.370 | 0.263–0.577 | 0.137–0.366 |

0.158 for *A. flinckii,* 0.044–0.094 for *A. guatemalensis,* 0.073–0.135 for *A. hickeli,* and 0.008–0.235 for *A. religiosa*), providing further evidence for the possible action of genetic drift on some of these populations.

The $F$ and $F_{it}$ values we observed suggest inbreeding in the analyzed populations. This was strongest for *A. guatemalensis,* but significant for all species. For *A. guatemalensis* and *A. hickeli,* values of $F_{it}$ (0.235 and 0.121, respectively) were even higher than values of $\theta$, indicating that a high portion of the total reduction in observed heterozygosity ($F_{it}$) is due to inbreeding within subpopulations, although interpopulational differentiation is also considerable, especially for *A. guatemalensis.* $F_{it}$ was also high in *A. religiosa* (0.216), but lower than $\theta$. While most conifers studied have displayed an excess of heterozygosity over that expected under Hardy-Weinberg equilibrium, heterozygote deficits have been reported for some predominantly outcrossing species (Parker and Hamrick, 1996), including *Abies* (Shea, 1990; Fady and Conkle, 1993; Table 3).

Estimates of $\theta$ for the populations analyzed show higher amounts of genetic differentiation among populations within species than that found for most other conifers and other species of *Abies.* This should not be surprising given the general bias in the literature toward studies of widespread, economically important conifers of the Northern Hemisphere. Mean $\theta$ values were 0.271 for *A. flinckii,* 0.122 for *A. guatemalensis,* 0.073 for *A. hickeli,* and 0.250 for *A. religiosa.* The relatively lower value for *A. hickeli* may be due to its more restricted geographic distribution, with all but one of the populations occurring in central Oaxaca. Hamrick and Godt (1996a) reported a mean $F_{st}$ of 0.101 for a variety of species with wind-dispersed pollen, and Hamrick, Godt, and Sherman-Broyles (1992) reported mean $F_{st}$ values of 0.063 for seven species of *Abies* and 0.073 for a variety of gymnosperm species.

The relatively high values of $\theta$ for the *Abies* species of southern Mexico and Guatemala reflect spatial genetic structure and suggest that genetic drift or selection or both have been important forces in their evolution. These results also suggest restricted levels of gene flow. The estimated levels of gene flow ($Nm$) in these species are lower than those needed to counteract genetic drift ($Nm > 4$; Jorgensen and Hamrick, 1997) and are lower than the mean values reported for other outcrossing species with wind-dispersed pollen (Hamrick, 1987).

Gene flow in *A. religiosa* is somewhat geographically restricted, with long-distance dispersal not sufficiently common to prevent isolation by distance. The sampled populations have likely existed for a long time, sufficient to permit isolation by distance to become apparent (Slatkin, 1993). The failure to detect an isolation by distance pattern in the other species could be due to continuing gene flow among populations, such as in the case of *A. hickeli* ($Nm = 3.17$), or due to the populations not being at drift-migration equilibrium because of historical events, such as recent range expansion or fragmentation of populations (Comes and Abbot, 1998). Gene flow may have been so restricted between even the closest populations within *A. flinckii* and *A. guatemalensis* that the regression between $M$ and distance was not sensitive enough to detect a pattern of isolation by distance. Also,

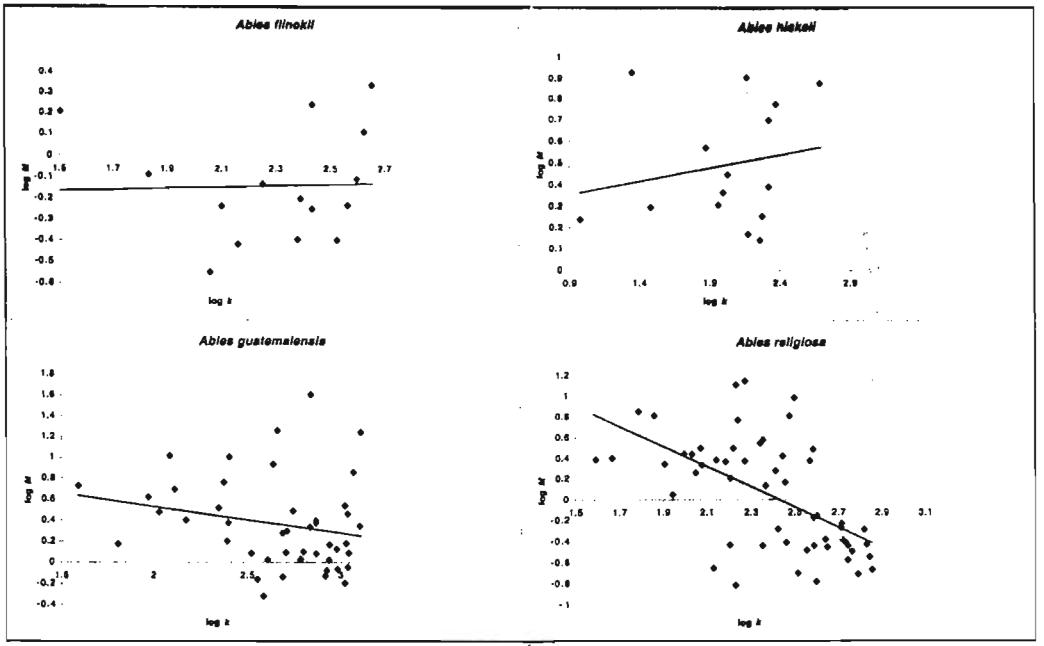


Fig. 2. Plots of all pairwise $\log_{10}M$ ($M$ = gene flow between pairs of populations) values against $\log_{10}k$ ($k$ = geographic distance between pairs of populations). The linear regression equations and probabilities ($p$) of obtaining these relationships given the null hypothesis of no relationship between $\log_{10}M$ and $\log_{10}k$ are, respectively, $\log_{10}M = 0.0298 \log_{10}k - 0.207$ and $p = 0.842$ for *Abies flinckii*, $\log_{10}M = -0.253 \log_{10}k + 1.036$ and $p = 0.537$ for *A. guatemalensis*, $\log_{10}M = 0.122 \log_{10}k + 0.246$ and $p = 0.881$ for *A. hickeli*, and $\log_{10}M = -0.974\log_{10}k + 2.366$ and $p = 0.0053$ for *A. religiosa*.

the very scattered and isolated populations of *A. guatemalensis* may not meet the assumption of the isolation by distance model.

Our phenogram provisionally suggests the existence of three major groups, *A. guatemalensis* (including *A. flinckii*), *A. hickeli*, and *A. religiosa*. We must caution, however, that the bootstrap confidence levels in this tree are low, likely due to the low levels of variation in the species, and further data from more variable markers will be necessary to draw firm phylogenetic conclusions.

While the populations of *Abies flinckii* form a monophyletic group, they are embedded within the *A. guatemalensis* group, suggesting that these may comprise a single species, as suggested by Martínez (1948) and Farjon (1990). This would not be entirely surprising, given the geographic distributions (Fig. 1) of the two species, but the great differences in phenology we observed between these species in the field suggest that they may be reproductively isolated. The clear difference between *A. flinckii* and *A. religiosa* is most strikingly apparent in populations F20 and R21, which represent trees mixed together on the same site in the Sierra de Manantlán. Although growing on the same site, their morphologies and phenologies are very different and no intermediates are found.

Branch lengths suggest that the populations of *A. flinckii* and the westernmost populations of *A. religiosa* (R21, R22, and R55) have undergone the highest degree of differentiation. These populations are quite isolated geographically and are not very large, conditions that may have permitted accelerated differentiation due to genetic drift. The relatively high θ value for *A. religiosa* may be due, in part, to the divergence between the western (e.g., R15, R21, R22, R49, and R53) and eastern (e.g., R45 and R48) populations that we observed in the tree.

All three of the populations that did not cluster with their conspecific populations (G44, G52, and H46) fall clearly within the reported geographic range of *A. religiosa*. The vegetative morphological differences between these populations and *A. religiosa* are not great and the species may have been misidentified by those who originally described these sites. In our field observations of vegetative characteristics, these three populations differed from *A. religiosa* principally in having leaves that were emarginate at the apex instead of acute, obtuse, or rounded. None of the populations showed evidence of being a mixture of species or hybrids. These populations are quite geographically isolated from their conspecific populations, and it is possible that they have undergone a very high degree of genetic drift and are now very different from the other populations in their species.

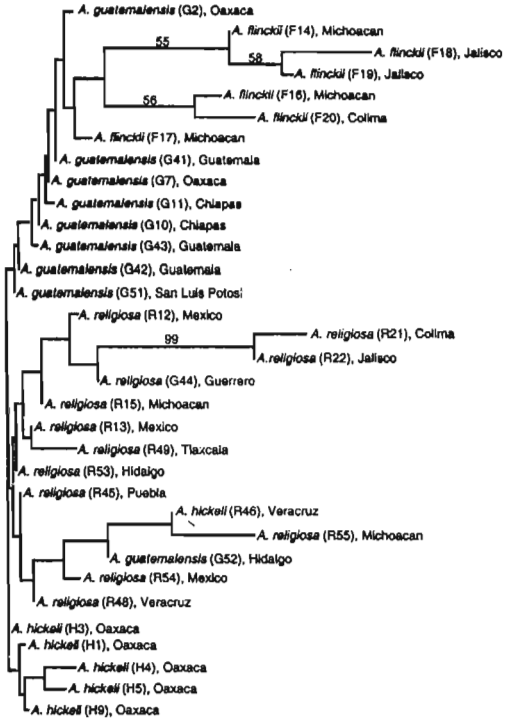


Fig. 3. Neighbor-joining dendrogram based on Nei's unbiased genetic distances between populations of *Abies flinckii* (F), *A. guatemalensis* (G), *A. hickeli* (H), and *A. religiosa* (R). See Fig. 1 for population locations. Numbers are bootstrap values (in percentages), values below 50 are not shown.

Population H4 corresponds to the type site for *A. zapotekensis* Debreczy, Rácz, and Ramírez, a new species described by Debreczy and Racz (1995). Our data suggest that it is a population of *A. hickeli*. Population R22 corresponds to the type site of *A. colimensis* Rushforth & Narave, a new species described by Rushforth (1989). Our data suggest that it is a population of *A. religiosa*, although the branch length suggests that it is a relatively more differentiated population. Population G10 corresponds to the type site of *A. guatemalensis* var. *tacanensis* (Lundell) Martínez. Our data suggest that it is a population of *A. guatemalensis*.

The short branch lengths separating the three major groups in the phenogram are consistent with the low amount of differentiation accounted for by differences among species (0.056). This low level of interspecific differentiation may be considered somewhat surprising, but high levels of genetic drift and low levels of gene flow among populations within species could generate high levels of intraspecific differentiation without necessarily leading to high levels of interspecific differentiation.

The effect of present geographic distribution on the patterns of genetic structure is not entirely clear. We found lower levels of genetic variation in *A. guatemalensis* than in the rest of the taxa, which was expected because of its smaller and more isolated populations. We also found indications of higher levels of inbreeding in this species. In contrast to what we expected, levels of interpopulational differentiation were higher in *A. religiosa* than in *A. guatemalensis*, although in both cases they were rather high.

In temperate species, cycles of glacial and interglacial periods likely have played an important role in shaping the present genetic variation patterns of plant populations (Konnert and Bergmann, 1995). The last glacial period reached a peak between 25 000 and 18 000 yr ago (Roberts, 1998). During this period, land temperatures dropped as much as 20°C and ocean temperatures ~5°C. These climatic changes led to changes in the distributions of many species, with species of the Northern Hemisphere displaced southwards. Afterwards, when warming

TABLE 3. Percentage polymorphic loci (P), mean expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), number of alleles per locus ($A$), Wright's fixation index ($F_{is}$) and total genetic diversity among populations ($G_{st}$ or $F_{st}$) for different species of *Abies*.

| Species | P[a] (%) | $H_e$ | $H_o$ | $A$ | $F_{is}$ | $F_{st}$ | Reference |
|---|---|---|---|---|---|---|---|
| *A. balsamea* | | 0.274 | 0.266 | 2.05 | −.008 | | Neale and Adams, 1985 |
| *A. fraseri* | 30.8 | 0.286[b] | 0.258[b] | 1.10 | 0.007 | 0.002 | Diebel and Feret, 1991 |
| *A. alba* | | | 0.397 | | 0.006 | 0.071 | Breitenbach-Dorfer et al., 1997 |
| *A. alba* | 54.5 | 0.182 | 0 149 | 1.6 | | | Fady and Conkle, 1993 |
| *A. cephalonica* | 72.7 | 0.221 | 0.161 | 2.0 | 0.234 | 0.048 | Fady and Conkle, 1993 |
| *A. borisii regis* | 59.1 | 0.198 | 0.161 | 1.8 | | | Fady and Conkle, 1993 |
| *A. lasiocarpa* | 43.4 | 0.124 | 0.081 | 1.6 | 0.341 | 0.017 | Shea, 1990 |
| *Abies* from the eastern U.S. | 42.1 | | | 1.4 | | | Jacobs, Werth, and Guttman, 1984 |
| *A. kawakamii* | 78 | 0.283 | 0.033 | 2.2 | | | Kormutak and Yang, 1998 |
| *A. hickeli* | 28.2[c] | 0.100 | 0.088 | 1.5 | 0.121 | 0.021 | This study |
| *A. guatemalensis* | 20[c] | 0.069 | 0.053 | 1.38 | 0.235 | 0.122 | This study |
| *A. religiosa* | 31.8[c] | 0.108 | 0.082 | 1.5 | 0.216 | 0.250 | This study |
| *A. flinckii* | 30.2[c] | 0.113 | 0.102 | 1.6 | 0.074 | 0.271 | This study |

[a] A locus was considered polymorphic if more than one allele was detected.
[b] Based only on polymorphic loci.
[c] A locus was considered polymorphic when the frequency of the most common allele was ≤95%.

began in the current interglacial period (~11 500 yr BP), species migrated northwards (Roberts, 1998).

The genus *Abies* has a long history in Mexico, with pollen present in Mexico at least as far south as Veracruz (Paraje Solo Formation), in the middle Pliocene (five million years ago; Graham, 1993, 1999). While we can estimate the current total sizes of these populations, we do not know their effective population sizes nor their historical sizes, both factors that can influence observed levels of variation. There are no detailed climatic data for Mexico during the glacial periods, but in general terms there is consistency in the data regarding the cold and dry climates during the glacial maximum (~18 000 yr BP; Lozano-García, 1993). It is proposed that between 12 500 and 9000 yr BP, at the end of the Pleistocene, the climate was humid and colder than at present and forests reached their maximum development in Mexico (Lozano-García, 1993). This suggests that during the last glaciation temperate species had a broader distribution than at present in Mexico, and populations of species that are now isolated could have been in contact. After 9000 yr BP, the climate became warmer (Lozano-García, 1993), and populations could have been fragmented, becoming smaller and more isolated, with reduced effective population sizes ($N_e$).

The *Abies* species of southern Mexico and Guatemala may have experienced fragmentation of populations and range retractions with the warming trend that peaked ~6000 yr BP. Their populations could have been displaced to higher regions, possibly causing a greater degree of isolation between populations than we observe at present. These populations may have passed through a number of genetic bottlenecks that led to a loss of genetic diversity and interpopulational differentiation due to genetic drift. Small population sizes could also have led to inbreeding and the observed heterozygote deficiencies (Nei, Maruyama, and Chakraborty, 1975). A number of the populations that we sampled have more than 100 individuals, which should be large enough to counteract genetic drift if all individuals reproduce, but *Abies* species are long-lived perennials with reproductive maturity at 20 yr and an average life span of 60 yr (Jacobs, Werth, and Guttman, 1984). If new variation is accumulated in proportion to the number of generations (meiotic events) over time, then these species may not have had enough time to recover the variation that they lost due to genetic drift.

The majority of conifers studied by isozyme analysis display relatively high levels of genetic diversity and low levels of interpopulational differentiation compared to other groups of plants. They also generally display genotypic frequencies consistent with Hardy-Weinberg equilibrium or an excess of heterozygotes. These conifers are mostly species with relatively large ranges in the North Temperate zones of Asia, North America, north of Mexico, and Europe. These areas were heavily impacted by Pleistocene glaciation, with much of their areas covered by ice. The current distributions of many of these species are very recent, not giving them enough time to develop high levels of interpopulational differentiation. Many of them were able to maintain relatively large continuous populations south of the glacial front during the Pleistocene, avoiding drastic losses of variation due to genetic drift. Their current large, often continuous populations help to maintain variation and prevent interpopulational differentiation and inbreeding.

In contrast, we observed relatively low levels of genetic diversity and relatively high levels of heterozygote deficiency and interpopulational differentiation in the *Abies* species of southern Mexico and Guatemala, suggesting the action of genetic drift and relatively low levels of gene flow. These same patterns were also observed by Keiman-Freire (1997) in a study of the *Abies* species of northern Mexico. *Picea chihuahuana,* another conifer with relatively small and isolated populations in northern Mexico, displayed the same pattern, with a low level of variation ($H_e$ = 0.093), and high levels of heterozygote deficiency ($F_{is}$ = 0.185) and interpopulational differentiation ($F_{st}$ = 0.248) (Ledig et al., 1997). *Pinus rzedowski,* a conifer with a small number of populations in a restricted area of west-central Mexico (western Michoacán) displays considerably higher within-population diversity ($H_e$ = 0.219), but also has a significant deficiency of heterozygotes ($F_{is}$ = 0.247) and a high level of interpopulational differentiation ($F_{st}$ = 0.175; Delgado et al., 1999). These data suggest that the levels and patterns of genetic variation observed for conifers in the North Temperate zone (Hamrick, Godt, and Sherman-Broyles, 1992) are not consistent with those of the conifers of Mexico studied until now. Ge et al. (1998) also reported very high levels of interpopulation differentiation in *Cathaya argyophylla,* a conifer with a restricted distribution in southern China. Hamrick and Godt (1996b) pointed out that, not only life-history traits, such as those shared by conifers, influence the levels and distribution of genetic diversity among species, but also the phylogenetic, biogeographical, and evolutionary histories of the particular species are important.

From the standpoint of conservation, the high levels of interpopulation genetic differentiation we observed pose a real challenge. This may be the case for many Mexican conifers and much of the Mexican flora. Other isozyme studies of Mexican plant species have found relatively high levels of interpopulation differentiation (Izquierdo and Piñero, 1998; Núñez-Farfán et al., 1996; Cornejo-Romero, 1998; Martínez-Palacios, Eguiarte, and Furnier, 1999). This pattern of variation poses the challenge of preserving the many genetically distinct populations necessary to adequately capture the genetic variation of the species, within a country with strong social pressures to economically exploit the land (Carabias, Arriaga, and Cervantes, 1994).

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**Phylogeography of *Abies* from southern Mexico and Guatemala: evidence of isolation by distance.**

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Keywords: *Abies hickeli*, *A.guatemalensis*, *A. religiosa*, *A. flinckii*, cpSSRs, phylogeography, ancestral polymorphisms.

**Abstract**

Paternally inherited polymorphic chloroplast microsatellites (cpSSRs) were used to examine genetic variation and to infer geographical distribution of pollen in 19 populations of *Abies* (eight of *Abies guatemalensis*, four of *Abies religiosa*, four of *Abies hickeli* and three of *Abies flinckii*) from southern Mexico and Guatemala. Populations of these species are distributed in mountain islands, some of them separated by many kilometers. A total of fifty-one different haplotypes were detected based on two cpSSRs, and high levels of haplotype diversity ($H_e$) were found (0.95 for *A. hickeli*, 0.89 for *A. religiosa*, 0.87 for *A. guatemalensis*, and 0.76 for *A. flinckii*). We used an analysis of molecular variance (AMOVA) to determine the hierarchical genetic structure of cpSSRs (88.4% variation within populations, 5.50% differentiation among populations of the same species and 5.66% differentiation among species), a neighbor-joining phenogram and a nested-clade distance analysis to provide insights into the evolutionary history of the species. Our results indicate that pollen could be the primary agent of gene flow ($F_{ST}$= 0.131 for *A. guatemalensis*, 0.075 for *A. religiosa*, 0.065 for *A. hickeli* and 0.029 for *A. flinckii*), although isolation by distance in certain populations seems to be promoting differentiation. The nested clade analysis suggests allopatric fragmentation in one lower-level clade, population expansion in one clade and isolation by distance in some lower and higher-level clades. Our data suggest that the *Abies* species studied are recently derived and are still not clearly differentiated at the molecular level, a finding that is in accordance with our allozyme data and with the hypothesis that their populations became isolated during the period following the last Pleistocene glaciation (6 000 yr BP).

## Introduction

The southernmost recognized species of *Abies* in America are *A. guatemalensis*, *A. religiosa*, *A. hickeli* and *A. flinckii* (Rushforth 1989; Aguirre-Planter et al. 2000). These species occur from San Luis Potosí in Mexico, to Guatemala. The phylogeography of southern species of *Abies* seems to be profoundly influenced by the warming period that followed the Pleistocene glaciation (Aguirre-Planter et al. 2000). During the last glaciation (~20 000 yr BP), climates were cooler and drier in Mexico (Lozano-García 1993), which could have favored the expansion of *Abies*. There is paleobotanical evidence that between 12 500 and 9 000 yr BP, at the end of the Pleistocene, temperate forests reached their maximum development in Mexico (Lozano-García 1993, Stute et al. 1995, Vázquez-Selem y Heine 2004). This information suggests that plant species of temperate forests had a broader distribution during the last glaciation than at present, and populations of *Abies* species from Mexico could have been in greater contact than at present (Aguirre-Planter et al. 2000). In the warming period after 9000 yr BP (including the present), as temperatures rose, the distribution of temperate species contracted since their habitats became more restricted and fragmented. Currently in Mexico and Guatemala, *Abies* species occur as isolated populations in mountain-top regions, often separated by many kilometers.

In a previous study we found low levels of genetic variation within populations and high levels of genetic differentiation among populations of southern species of *Abies* species at allozyme loci, suggesting that gene flow is limited and the random loss of alleles through genetic drift is relatively high (Aguirre-Planter et al. 2000). This pattern contrasts to what has generally been found in conifers of the northern temperate zone (Hamrick et al. 1992; Ledig 1998). However, high levels of genetic differentiation among populations have also been found in other conifer species in Mexico and Central and South America (Ledig et al. 1997; Allnutt et al. 1999; Matheson et al. 1989; Delgado et al. 1999; Molina-Freaner et al. 2001), a result that has similarly been attributed to the mountainous topography and associated to climatic heterogeneity.

Gene flow in plants is mediated by pollen and seed dispersal. Since in conifers chloroplasts are primarily paternally inherited (Wagner 1992) and mitochondria maternally inherited, analyses of plastid genes provide information about the effects of male and/or female gene flow (Ennos 1994). In particular, comparing nuclear with chloroplast genetic markers allows inferences about pollen dispersal (McCauley 1995; Latta & Mitton 1997; Viard et al. 2001).

Microsatellite or SSR (Simple Sequence Repeat) markers in conifer chloroplast DNA (in both *Pinus* and *Abies*) have been useful for inferring population genetic processes because of their relatively high mutation rates (Powell et al. 1995; Vendramin et al. 1996; Vendramin and Ziegenhagen 1997). Since the effective population size of chloroplast markers is approximately one-half of that of diploid nuclear markers in hermaphroditic, outbreeding plant species (McCauley 1995), plastid markers are more sensitive indicators of bottleneck effects and genetic drift. Chloroplast SSRs can also be used to study lower (species/subspecies) taxonomic categories, since the chloroplast genome is assumed to be non-recombining (Clegg 1993), and they appear to have lower mutation rates than nuclear microsatellites, and it, leading to fewer errors in analyses due to homoplasy (Provan et al. 1999a). Homoplasy occurs when different copies of a locus are identical in state, although not identical by descent and it is related to the way mutation produces new alleles (Estoup et al. 2002). The mutational models generally proposed for microsatellites are the stepwise mutation model (SMM, Kimura & Ohta 1978), the infinite allele model (IAM, Kimura & Crow 1964) in which homoplasy is not expected, and the two-phase model (TPM, Di Rienzo et al. 1994). Homoplasy can affect the inferences drawn in a phylogeographic study.

In species with long generation times, such as conifers, cpSSRs markers have allowed the delineation of related groups. For instance, Bucci et al. (1998) used cpSSR markers to distinguish closely related taxa of the *halepensis*-complex of pine species, whereas Clark et al. (2000) were able to differentiate between *Abies fraseri*, *A. balsamea* and *A. balsamea* var. *phanerolepis*. In both cases differentiation was not possible using only allozyme data alone. Gugerli et al. (2001) found congruent

phylogenetic relationships among *Pinus cembra*, *P. sibirica*, and *P. pumila*, based on cpDNA microsatellites and mitochondrial nad1 intron 2 sequences.

Using molecular markers such as cpSSRs enables us to detect phylogeographic patterns and population genetic structures. The study of geographical subdivisions and the historical processes that gave rise to them can be studied in detail by using a nested-clade phylogeographical analysis proposed by Templeton et al. (1995). This method defines a series of hierarchically nested clade using explicit nesting rules (Templeton et al. 1987, Templeton et al. 1992). Haplotypes are nested together in one-step clades, based on mutational steps. The one-step clades are nested together in two-step clades, and so on, until the last nesting level comprises the entire haplotype network. Based on the significant association between haplotype/clades and geography and on the NCPA inference key (Templeton, 2004) one can discriminate among population processes such as gene flow, from historical events such as range expansions, long distance colonization and fragmentation, that gave rise to the current configuration of population/haplotype variation (Templeton 2001).

In this study we used chloroplast microsatellite (cpSSRs) markers to examine the extent of genetic variation and the structure of current populations in *A. guatemalensis*, *A. hickeli*, *A. religiosa* and *A. flinckii*. We are particularly interested in addressing the importance that pollen flow and historical population events have had in shaping the current structure of populations within and among taxa. We are also interested in delimiting the taxonomic boundaries between the four taxa at the molecular !evel, since this was not possible in our previous allozyme study (Aguirre-Planter et al. 2000). Finally, by combining the information from this and our previous study, we propose conservation strategies for the genetic resources in each taxon.

## Materials and methods

### *Species description*

Four species of *Abies* from southern Mexico and Guatemala were studied: 1*) Abies guatemalensis* Rehder, the southernmost representative of the genus, which is found

in Guatemala, and the Mexican states of Chiapas, Oaxaca, Guerrero, Jalisco, Hidalgo and San Luis Potosí (Martínez 1948; Donahue et al. 1985). *A. guatemalensis* was considered a common species until 1940, but since then it has been widely exploited throughout its range and it is currently considered an endangered species. Wood of *A. guatemalensis* is locally used as construction material and as firewood and forests are cleared because of their fertile soils that have been used for agriculture and pasture. At the present time, although this species is widely distributed, it has relatively few isolated populations, and has consequently been designated as a threatened with extinction species (FAO 1986; CITES Appendix 1, http://www.cites.org/, included by the Mexican government in the NOM-ECOL-059-1994). 2) *Abies religiosa* (H. B. K.) Schl. et Cham. grows throughout the Transversal Volcanic Belt in the states of Guerrero, Jalisco, Michoacán, México, Morelos, Hidalgo, Tlaxcala, Puebla, Veracruz and Distrito Federal (Martínez 1948). Its distribution is more continuous than *A. guatemalensis* and its forests are sanctuaries for the monarch butterfly during ivernation. 3) *A. hickeli* Flouss et Gaussen, which has a restricted distribution, with only a few populations in the states of Oaxaca and Veracruz (Martínez 1948). The wood of this species is locally used for house and furniture construction and because of its extraction and depletion of populations, it is considered now as a threatened species by the Mexican government (NOM-ECOL-059-1994); and finally 4) *A. flinckii* Rushforth, which is distributed in Jalisco and Michoacán. *A. flinckii* was originally considered to be either *Abies religiosa* var. *emarginata* Martínez or *A. guatemalensis* var. *jaliscana* Martínez (Martínez 1948). It is currently considered as a species that requires special protection (NOM-ECOL-059-1994).

Farjon (1990, 1998) used morphological characters to classify and divide the genus *Abies* in Mexico into different sections: Section *Grandis* Engelm. Emend. Farjon et Rushforth, including *A. grandis* (a species not found in Mexico), *A. concolor, A. durangensis, A. guatemalensis* and; Section *Oyamel* Franco, Subsection *Religioseae* (Matzenko) Farjon et Rushforth which comprises *A. religiosa* and *A. vejarii* and Subsection *Hickelianae* Farjon et Rushforth which includes only *A. hickeli. A. flinckii* is not considered as a species in Farjon's classification, since it was

previously considered as two taxa: *Abies religiosa* var. *emarginata* and *A. guatemalensis* var. *jaliscana*.

Samples from 19 populations were selected for this study: eight populations of *A. guatemalensis*, four of *A. religiosa*, three of *A. flinckii*, and four of *A. hickeli* (Fig. 1). Sample sizes analyzed ranged from 13 to 29 trees per population (Table 1). Populations chosen are a subset of those investigated in a previous allozyme study (Aguirre-Planter et al. 2000).

*DNA extraction and PCR analysis*

Polymorphism at eight chloroplast microsatellite loci (Vendramin & Ziegenhagen 1997) was analysed, but only two of them were polymorphic, the same loci that have been found to be polymorphic in different species of *Abies* (Vendramin and Ziegenhagen 1997; Clark et al 2000). Microsatellite Pt 30204 is composed of three mononucleotide repeats and it is located in a protein gene, *clpP* (location refers to *Pinus thunbergii*), and microsatellite Pt 71936, which contains only a mononucleotide stretch of thymidine repeats and it is located in an intron containing reading frame, *IRF169* (location refers to *Pinus thunbergii*) (Tasuya et al. 1994, Vendramin and Ziegenhagen 1997). Total DNA was isolated from leaf samples of all individuals using a CTAB mini-prep protocol (Vázquez-Lobo 1996). PCR amplifications were performed in a MJR PTC-100 thermal controller using approximately 25 ng of genomic DNA in a 15 μL volume reaction containing 10X *Taq* salts buffer (100 mM Tris-HCL pH 8.4, 500mM KCl, 60 μL of BSA (100mg/ml) and gelatin 1%), 0.2 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.2 μM of each primer (one of the primers was end-labeled with infrared IRD dyes for visualization with a Li-Cor automated sequencing system) and 1 U of *Taq* polymerase. Amplification was carried out for an initial period of denaturation at 95°C for five min followed by 25 cycles of: 1 min of denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C, with a final extension step at 72°C for 8 min. Five μL of formamide loading dye were added to each reaction and the samples were denatured at 80°C for 3 min prior to electrophoresis.

The PCR products were run on a 14%, 18 cm long denaturing polyacrylamide gel on a Li-Cor 4000L automated sequencer set at 50°C, 1000V, 35 mA and 25W. A 16-bit image of each gel was stored after each run. Infrared IRD-labelled size standards in the outermost and middle lanes were loaded on the same gel, as well as control samples of known size in each run. The size of each DNA fragment was estimated using the program RFLPSCAN 3.0 (SCANANALYTICS). This program assigns molecular weights to fragments by comparing the size of fragments in experimental samples to molecular weight standards and control individuals.

*Data Analyses*

The haplotype identity of each individual was established considering both loci. The number of haplotypes and haplotype frequencies were estimated for each population. Genetic diversity within populations was calculated as $H_e = [n/(n-1)](1-\Sigma p_i^2)$, where $p_i$ is the frequency of the ith-haplotype and n is the number of individuals analysed (Nei 1987).

Population structure was assessed with the stepwise-mutation model, using $R_{ST}$ (Slatkin 1995), which theoretically is a more accurate estimate for describing population structure based on SSR data, and with the infinite allele model, with $F_{ST}$ (Wright 1965). In theory, $R_{ST}$ performs better than $F_{ST}$ under ideal conditions (large sample sizes and many loci), but when there are few loci and/or the divergence within the group is low, $R_{ST}$ can lead to a serious bias in the estimation of gene flow and perform more poorly than $F_{ST}$ (Gaggiotti et al. 1999).

A hierarchical analysis of molecular variance (AMOVA, Excoffier at al. 1992) was performed in order to partition genetic divergence among regions, among populations within regions, and within populations ($\phi_{CT}$, $\phi_{SC}$ and $\phi_{ST}$ respectively). The significance of the obtained values was tested using a non-parametric permutation approach described in Excoffier et al. (1992). All of the analyses above were carried out using Arlequin v. 2000 (Schneider et al. 2000).

Estimates of gene flow were obtained from mean estimates of $F_{ST}$ and $R_{ST}$ as $Nm = 1/2\ [(1/F_{ST}) - 1]$ (Wright 1951). To test for isolation by distance, the correlation between gene flow ($M$) between pairs of populations and their geographic distance

were estimated for each species (Slatkin 1993). The significance of the correlations was tested by a Mantel test (Mantel 1967).

We constructed a neighbor-joining tree using PHYLIP (Felsenstein 1995), based on two distance matrices, Nei's unbiased genetic distance ($D_A$) (Nei 1978), and the stepwise distance ($D_{SW}$) of Shriver et al.(1995). We used the nested-clade analysis described in Templeton et al. (1987, 1992) to construct a haplotype network with a nested design, using the program TCS, version 1.13 (Clement et al. 2000). The nesting algorithm (Templeton et al. 1987) groups together haplotypes and clades by the number of mutational changes, separating them until all haplotypes/clades are joined and partitioned in 1, 2 or more mutational step groups. The final nesting level comprises the entire network. The relationship between haplotypes/clades is then examined by performing exact permutation contingency tests using GEODIS 2.0 (Posada et al. 2000), in order to evaluate if there are significant associations between geographic and haplotype/clade positions at each level of nesting. In clades where a significant association was found, a distance analysis was performed using GEODIS 2.0 (Posada et al. 2000) and the results interpreted using the inference key by Templeton (2004), which gives criteria to discriminate between the role that fragmentation events, gene flow, isolation by distance, population range expansion and/or long distance colonisation events have played in the historical processes that resulted in the current relationships among haplotype/clades.

## Results

*Genetic variation*

Ten different size variants were found at locus Pt 71936 and 15 at locus Pt 30204. Combining the size variants at both loci produced a total of 51 different haplotypes (Table 2). Some of the populations had unique haplotypes, such as AG11 (2 unique haplotypes), AG41 (3 unique haplotypes), AH3 (2 unique haplotypes), AH47 (1 unique haplotype), R22 (2 unique haplotypes) and AF18 (2 unique haplotypes). Populations of *A. flinckii* had many unique haplotypes (7), while the other three species (*A. guatemalensis*, *A. hickeli* and *A. religiosa*) shared 20 haplotypes. Mean $H_e$ was high for each of the four species examined (0.95 for *A. hickeli*, 0.89 for *A. religiosa*, 0.87 for *A. guatemalensis* and 0.76 for *A. flinckii*; Table 1). There were on average 11 haplotypes in populations of *A. hickeli*, 10.37 in populations of *A. guatemalensis*, 9.5 in populations of *A. religiosa* and 6.33 in populations of *A. flinckii*. Population G51 had the lowest diversity ($H_e$= 0.566, 4 haplotypes); it is the most northern and isolated population of *A. guatemalensis*, and harbours one common haplotype with high frequency (Table 2) and three other haplotypes with low frequencies.

*Genetic differentiation*

The $F_{ST}$ estimates indicate that there is lower differentiation among populations of *A. flinckii*, intermediate for *A. hickeli* and *A. religiosa*, and higher for *A. guatemalensis* (Table 3). On the other hand, $R_{ST}$, estimates indicate lower differentiation within *A. hickeli*, intermediate for *A. guatemalensis* and *A. flinckii* and higher for *A. religiosa* (Table 3). No significant correlation between estimates of $F_{ST}$ and $R_{ST}$ was found. Relatively high estimates of gene flow were obtained for the taxa studied (Table 3). The highest gene flow estimates were for *A. flinckii* (*Nm*= 16.74) and the lowest for *A. guatemalensis* (*Nm*= 3.32; Table 3). Caution should be taken with these estimates, since the analysis assumes that $F_{ST}$ values are in equilibrium, and these values could be overestimates (see below). Correlations between the logarithm of pairwise gene flow (log $MF_{ST}$) and the logarithm of geographic distance between pairs of populations (to test for isolation by distance)

were only significant using $F_{ST}$ for *A. guatemalensis* ($r$ = -0.4690, $p$ = 0.002) (Fig. 2). This species does have the widest distribution (Fig 1). For $R_{ST}$ none of the correlations were significant with any of the species.

Partitioning of genetic variation using AMOVA (Excoffier et al. 1992) shows that 88.4% of the total variation is due to variation within populations; differentiation among populations of the same species was only 5.50% and between species only 5.66%. Estimated values of differentiation among species was $F_{ST}$= 0.120 and $R_{ST}$= 0.204.

*Phylogenetic analyses*

The phenogram constructed with Nei's unbiased genetic distance ($D_A$) (Fig. 3) provides better resolution than the phenogram constructed using Shriver's distance ($D_{SW}$) (not shown), and therefore the discussion will be based only on the former. Both phenograms show a unique group for *A. flinckii*, but they do not discriminate well among the other three species (*A. guatemalensis*, *A. religiosa* and *A. hickeli*). There are three main groups in the phenogram. I) One including populations of *A. guatemalensis*, *A. religiosa* and *A. hickeli*, all in Mexico, although embedded in this group are three populations of *A. guatemalensis* {San Luis Potosí (G51), Chiapas (G11) and Guerrero (G44)} that are separated by longer terminal branches. II) A second group consisting only of populations of *A. guatemalensis* from Guatemala (G41 and G42). III) A third group consisting of all the populations of *A. flinckii*.

*Nested clade analysis*

The relationship between haplotypes/taxa is depicted in the nested haplotype network in Fig. 4. Clades 3-2 and 3-4 harbour many of the haplotypes, and moreover, many of them are shared between *A. guatemalensis*, *A. hickeli* and *A. religiosa*. The same situation also occurs, but to a lesser extent, in clade 3-1. Haplotypes 9, 10, 11, 12, 17, 18, 19 and 24 are the most common and widely distributed in populations of *A. guatemalensis*, *A. hickeli* and *A. religiosa*. Haplotypes in clade 3-3 are primarily from *A. flinckii*, with some haplotypes found in only one individual of another species. Clade 2-2 is disjoint from the other clades and harbours only rare haplotypes from *A. guatemalensis*. Clades outlined in color

show significant geographic-clade associations. Based on the inference key of Templeton et al. (1995), an allopatric fragmentation event was detected in one-step clade, 1-17 (outlined in blue). A pattern of restricted gene flow resulting from isolation by distance was detected in clades 1-9, 1-16, 1-23, 2-2, 2-7, 2-10 and 3-4 (outlined in red). Finally, range expansion was detected in clade 1-13 (outlined in green).

## Discussion

The geographical distribution of chloroplast DNA polymorphisms and levels of differentiation found among populations of each taxon suggest that pollen flow is relatively high. Distribution of ancestral shared haplotypes among populations of *A. guatemalensis*, *A. hickeli* and *A. religiosa* and the nested clade analysis suggest a recent diversification of these species.

### *Genetic variation*

A total of 51 different haplotypes were found in the 373 individuals analysed. This is a relatively high number of haplotypes identified using two chloroplast microsatellite loci. Haplotypic diversity was also high in the four taxa studied ($H_e$= 0.87 for *A. guatemalensis*, $H_e$= 0.95 for *A. hickeli*, $H_e$= 0.76 for *A. flinckii* and $H_e$= 0.89 for *A. religiosa*). Similar levels of haplotypic diversity at these two chloroplast microsatellite loci have also been reported in populations of *Abies alba*, *Abies fraseri* and *Abies balsamea* (Vendramin et al. 1999; Clark et al. 2000) (Table 4). In general, we would expect to find such high levels of diversity, since mutation rates are estimated to be relatively high at microsatellite loci (although exceptions to this pattern can be seen in Provan et al. 1999b). In other conifers such as *Pinus halepensis*, *P. pinceana* and *Pseudotsuga menziesii*, levels of haplotypic diversity ($H_e$) are lower (Table 4). The high levels of diversity found in this study and in other European and North American *Abies* species (Clark et al. 2000), is due to the high number of size variants found at microsatellite Pt 30204 and to the existence of a high proportion of the two locus combinations.

### *Genetic differentiation*

In both the island model and the isolation by distance model we expect a greater differentiation for paternally inherited chloroplast genes compared to biparentally inherited nuclear genes (Ennos 1994), since the effective population size of haploid organelle genes is one-half that of diploid nuclear genes (McCauley 1995). For instance, Vendramin et al. (1999) found higher or similar levels of genetic differentiation based on cpSSRs data ($G_{ST}$= 0.133) than previously found with allozymes in *A. alba* ($G_{ST}$= 0.095, Schroeder et al. 1989 and $G_{ST}$= 0.088, Bergmann

1994 in Vendramin et al. 1999). This pattern was also found in *Pinus pinceana* ($F_{ST}$ = 0.152 for allozymes, Ledig et al. 2001 and $F_{ST}$ = 0.785 for cpSSRs, Escalante 2001). Matos and Schaal (2000) also found higher levels of differentiation using chloroplast DNA markers in *Pinus hartwegii* and *P. montezumae*.

We also expected that small and isolated populations will be more affected by genetic drift at organelle loci than at nuclear genes, if rates of pollen and seed dispersal are similar. However, in our study we found that differentiation for paternally inherited genes was lower than that for biparentally inherited nuclear genes. $F_{ST}$ and $R_{ST}$ estimates for chloroplast microsatellites showed lower levels of differentiation than those found at allozyme loci in the same species (Aguirre-Planter et al. 2000), except for *A. guatemalensis*, where $F_{ST}$ estimated with cpSSRs ($F_{ST}$ = 0.131) is similar to that estimated with allozymes ($F_{ST}$= 0.122).

One possible explanation to this result is that chloroplast microsatellite data show us a different process from the allozyme data and indicate the importance of pollen flow in shaping the genetic structure of species. For instance, in *Pseudotsuga menziesii*, Viard et al. (2001) found significant among-population differentiation with allozyme data, but no differentiation among populations at cpSSR loci. They considered that this pattern is due to limited seed dispersal, relative to pollen dispersal. This could also be the case for the species of *Abies* we studied, since pollen in *Abies* is dispersed by wind and can travel long distances, while seeds are not expected to move as far as pollen because of their greater mass. Liepelt et al. (2002) showed that pollen-mediated gene flow has spanned a much greater distance than suggested from allozyme data in *Abies alba*. Hence, pollen may be the primary agent of gene flow between populations in the species studied.

Another possible explanation for the pattern of differentiation we observed is that mutation rates in microsatellite loci can be very high and this could lead to an underestimate of genetic differentiation based on F-statistics when migration is low (Hedrick 1999), which may be the case of the species studied. However, in a study with *P. torreyana*, Provan et al. (1999b) suggested that compared to nuclear microsatellites, chloroplast microsatellites have low mutation rates (ranging from $3.2 \times 10^{-5}$ to $7.9 \times 10^{-5}$), and it is unlikely that they are of the order of magnitude of

migration rates. Therefore, mutations at cpSSRs loci would not appear to occur at a level that would confound analyses of population genetic structure.

*Abies guatemalensis* displays a higher degree of differentiation among populations and lower levels of gene flow ($F_{ST}$= 0.131, $Nm$= 3.32) at cpSSRs loci, with a significant pattern of isolation by distance, probably resulting from reduced dispersal and consequent genetic drift. This would be expected, since *A. guatemalensis* has a patchy, wide distribution with many isolated populations (Fig 1). In contrast, *A. flinckii* shows no significant differentiation among populations, possibly due to its more restricted distribution, with the populations being closer to each other, allowing pollen dispersal to reduce population differentiation. Levels of differentiation and gene flow in *A. religiosa* and *A. hickeli* are intermediate ($F_{ST}$= 0.075, $Nm$= 6.09 and $F_{ST}$= 0.065, $Nm$= 7.19, respectively), and lower than that found for *A. alba* ($F_{ST}$= 0.133) (Vendramin et al. 1999).

*Genetic relationships among populations and species*

It has been argued that the analysis of genetic relationships among populations and species using microsatellite loci is more accurate when using stepwise mutation models (Goldstein et al. 1995), but, as it was mentioned before, when there are few loci and/or the divergence within the group is low, which is the case for our data, it can lead to serious bias in estimating genetic relationships (Gaggiotti et al. 1999). In our study, the phenogram constructed with Nei's distance ($D_A$) had a better resolution to distinguish among the four taxa studied than Shriver's distance ($D_{SW}$), which is based on a stepwise mutation model. Goldstein et al. (1995) reported that 500 generations are needed to detect population divergence using $R_{ST}$ at nuclear microsatellite loci. The slower mutation rate in cpDNA, coupled with a long generation time, means that a longer time is necessary to detect differentiation using the stepwise mutation model (Richardson et al. 2002). If we assume that *Abies* has a generation time of approximately 33 yr (Silvertown and Dodd 1999) and that populations of these species have been separated for approximately 9 000 yr (during the warmer period following the last glaciation), then only 272 generations have passed.

The branch lengths on the tree are short, with the exception of the *A. flinckii* group and populations G11, G44 and G51, which have undergone the highest degree of differentiation. Bootstrap values are low (<77), with the exception again of the node that separates *A. flinckii* and the one that separates populations of *A. guatemalensis* from Guatemala. This is consistent with the relatively low differentiation observed among species ($F_{ST}$= 0.120).

The phenogram suggests three major groups: One group containing populations of *A. guatemalensis, A. religiosa*, and *A. hickeli* in Mexico. Within this group, population G51 of *A. guatemalensis* exhibits the highest degree of divergence. This is the northernmost population of *A. guatemalensis*. It is markedly isolated and harbours relatively few mature trees, factors that could have caused it to have accelerated differentiation due to genetic drift. Population G51 might have been relatively recently founded causing it to have a high frequency of rare haplotypes due to random sampling of alleles. G44 also has a greater degree of divergence and it is also isolated, being the only population of *A. guatemalensis* in Guerrero. The second group clusters populations of *A. guatemalensis* from Guatemala (G41 and G42), apparently well differentiated from Mexican populations. A third group consists of populations of *A. flinckii* and shows species-specific haplotypes. We have also observed great differences in phenology in the field between *A. flinckii* and the other species, with the former beginning its vegetative growth as much as three months earlier. Even where trees of *A. flinckii* and *A. religiosa* are intermixed at the same site (Sierra de Manantlán), their phenology and their leaf morphology are very different, so we are confident that they can be considered distinct species.

The nested geographical distance analysis (NCA) allows to infer phylogeographic events. An observed pattern in the higher nesting clade 3-4, that contains presumably the oldest haplotypes, is restricted gene flow due to isolation by distance. This pattern is also inferred in clades at lower nesting levels (1-9, 1-16, 1-23, 2-2, 2-7 and 2-10). We also inferred an allopatric fragmentation event in clades 1-17 and one event of recent expansion in clade 1-13. The NCA does not detect lineages that correspond to each species, although it separates populations of *A. flinckii* in a different group. While *A. guatemalensis*, *A. hickeli* and *A. religiosa*

share most of their haplotypes, clade 3-3 includes mainly haplotypes from *A. flinckii*. Haplotypes in this clade that do not belong to *A. flinckii* are present in only one or two individuals from populations of the other taxa and they could be the result of homoplasy (but see below). Clade 3-3 could be considered a different species (*A. flinckii*) under the biological and phylogenetic species concept. Nevertheless, according to Templeton (2001), to infer a species under the cohesion species concept from a phylogeographic NCA we need to detect fragmentation. We did not detect a fragmentation event within the *A. flinckii* clade. However, our data overwhelmingly support the proposal that *A. flinckii* is a distinct species. Our inability to detect it here could be caused by a lack of statistical power, resulting from insufficient number of trees and/or populations sampled, or to the number of loci used. The NCA demonstrates that restricted gene flow due to isolation by distance has been very important in shaping the genetic structure of populations of these *Abies* species. This result is consistent with the hypothesis that the climate warming, following the Pleistocene glaciation, resulted in isolation of populations in mountain-top regions.

The existence of widespread common haplotypes for *A. guatemalensis*, *A. hickeli* and *A. religiosa* is a clear suggestion of interspecific introgression and/or shared ancestral polymorphisms that predate the divergence of these species. The difference in morphology and phenology between these species (data not shown) suggests that they are mostly reproductively isolated. Phylogeographic approaches, such as the NCA, can potentially discriminate between the effects of on-going gene flow and shared polymorphisms caused by ancestral polymorphisms (Templeton 1998, 2001). In the NCA the most ancient haplotypes are typically located at the centre of the nested network. In our analysis, the ancient haplotypes are shared by *A. guatemalensis*, *A. religiosa* and *A. hickeli*. They predate the divergence of species, so we may infer that their distribution owes more to the persistence of ancestral polymorphisms than to recent gene flow. We cannot be confident in the conclusions drawn from our phylogeographic inferences if homoplasy is recurrent in the cpSSRs loci in our *Abies* species. Estoup et al. (2002) found that the large amount of variability at microsatellite loci can compensate for their homoplasious

evolution and that homoplasy depends on different evolutionary factors such as mutation rate, the effective population size and the time of divergence between populations. Mutation rates in chloroplast microsatellites (on the order of $10^{-5}$) are lower than in nuclear microsatellites (Provan et al. 1999a). Estoup et al. (2002) found that with these mutation rates and effective population sizes of less than 1000 homoplasy is almost nonexistent. Levels of homoplasy increase with the time of divergence (Provan et al. 2001; Estoup et al. 2002). If we assume, as mentioned before, that the time of divergence between these species is at most 272 generations ago, it would also support our suggestion that levels of homoplasy may not be affecting the patterns we are finding. Also, when homoplasy is high, linkage disequilibrium should be low, since the chloroplast genome is nonrecombinant (Provan et al. 2001). We found considerable levels of linkage disequilibrium in some haplotypes (table 5), suggesting low levels of homoplasy. Homoplasy is not expected under the infinite allele model (Estoup et al. 2002). We tested alternate mutation models (Cornuet & Luikart 1996) and found that the loci that we used are well-described by the infinite allele model (data not shown).

There are other studies that have employed microsatellites to infer phylogeographic structure and historical events in different species. For instance, Reush et al. (2001) used microsatellite loci to evaluate the effects of different historical events on genetic divergence among populations of threespine stickleback (*Gasterosteus aculeatus*). They argued that homoplasy was of minor importance in their study, because of the young age of *Gasterosteus* populations (< 12 000 years). Cuenca et al. (2003) used microsatellite data to perform a nested clade analysis (NCA, Templeton et al. 1995) to infer population history in *Pinus nelsonii*, and Marshall et al. (2002) employed cpSSRs to investigate phylogeography of *Pinus contorta*.

By comparing the geographic distribution of populations (Fig.1) to the haplotype network (Fig. 4), it is clear that populations from the center of the distribution of *Abies* in Mexico and Guatemala (circled in the map), harbour the oldest haplotypes and that these haplotypes have persisted in populations of *A. guatemalensis*, *A. hickeli* and *A. religiosa*. Clearly, *A. flinckii* forms a different group. The completely different haplotypes for *A. flinckii*, along with their distinct polymorphic allozyme

loci and very different phenology (Aguirre-Planter et al. 2000) may be the result of an early isolation of its populations followed by rapid diversification, or it may be the result of a different introduction from the north (see below).

*Evolutionary history of the Southern Abies*

*Abies* species could have migrated to the south througout either of two routes: 1) the Sierra Madre Occidental in the west, possibly representing the ancestral introduction that gave rise to *A. flinckii*, or 2) the Sierra Madre Oriental in the east, that could have given rise to *A. guatemalensis*, *A. hickeli* and *A. religiosa*. Some authors propose that northern temperate taxa migrated southward into Mexico during periods of glaciation in the Pleistocene (Deevey, 1949; Dressler 1954 in Perry et al. 1998). Other authors have postulated that these taxa began migrating into Mexico and Central America during the early and middle Tertiary (Chaney 1936; Steyermark 1950; Braun 1955; Martin and Harrel 1957 in Perry et al. 1998). The first occurrence of *Abies* in the fossil record of Mexico is from the Paraje Solo assemblage in the middle Pliocene (five million years ago) (Graham 1999), so the divergence of these species may be a relatively recent event (on an evolutionary scale). These populations may not have been isolated for a long time, since the *Abies* species may have experienced range retractions and fragmentation of populations with the warming period that peaked ~6 000 yr BP (Aguirre-Planter et al. 2000) and *Abies* species have relatively long generation times. As we mentioned before, considering a generation time of 33 yr approximately, only 272 generations at the most have passed since these populations have been isolated.

Our cpSSRs analyses suggest that southern species of *Abies* are recently derived. Although they may not be currently interbreeding, they have diverged so recently that some populations may still be in the process of speciation and they still share extensive ancestral polymorphisms. The low levels of cpSSRs differentiation and relatively higher allozyme (nuclear markers) differentiation among populations of *Abies* in southern Mexico and Guatemala may be attributed to limited seed dispersal and greater pollen movement, although isolation by distance seems to be promoting differentiation. Nevertheless, the estimates of gene flow should be taken with

caution in these species, since populations are rather young and populations that were historically in contact and that are currently isolated could be eventually fixed for different alleles at equilibrium. The time required to reach equilibrium could be longer than the age of most of these species (Neigel 2002). Our analyses indicate that there are three clear genetic groups. One of *A. flinckii*, that has diverged more, another including populations of *A. guatemalensis* from Guatemala and a third one comprising *A. hickeli*, *A. religiosa* and *A. guatemalensis* from Mexico, in which we find ancestral haplotypes. We infer that populations in the central part of the distribution (H3, H4, H46, H47, R48, R49, G2, G7, G44) are probably the ancestral ones.

We consider that different kinds of molecular markers can give us a better picture of the overall history and resources of these species. This knowledge is a valuable tool for making decisions concerning the conservation or management of populations of the species. Previously, we concluded (Aguirre-Planter et al. 2000) that the high levels of genetic differentiation observed among populations suggested the importance of preserving many genetically distinct populations to conserve the genetic variation of the species. The cpSSR results suggest that we need to preserve populations from three distinct genetic groups and populations that have unique haplotypes like G41, G52, G7, R22 and H3. *A. guatemalensis*, a threatened species (FAO 1986; CITES, Appendix 1, http://www.cites.org/) has the highest levels of differentiation among populations and efforts must be focused on preserving its populations.

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Table 1. Geographic coordinates, elevation (m), number of individuals analyzed (N), number of haplotypes (Nh) and haplotype diversity ($H_e$) in populations of *Abies guatemalensis* (AG), *A. hickeli* (AH), *A. flinckii* (AF) and *A. religiosa* (AR). Standard errors are in parentheses.

| Population | Lat N/Long W | Elevation | N | Nh | $H_e$ |
|---|---|---|---|---|---|
| AG2 | 17°10'/96°30' | 2800 | 25 | 13 | 0.930 (0.029) |
| AG7 | 16°11'/96°18' | 2500 | 19 | 11 | 0.930 (0.034) |
| AG11 | 15°27'/92°16' | 2610 | 22 | 10 | 0.822 (0.073) |
| AG41 | 15°04'/89°55' | 2970 | 17 | 14 | 0.978 (0.027) |
| AG42 | 14°31'/90°08' | 2610 | 18 | 6 | 0.843 (0.045) |
| AG44 | 17°35'/99°51' | 2670 | 21 | 12 | 0.933 (0.031) |
| AG51 | 22°27'/99°27' | 1770 | 17 | 4 | 0.566 (0.123) |
| AG52 | 20°21'/98°20' | 2310 | 21 | 13 | 0.929 (0.039) |
| mean | | | 20 | 10.37 | 0.87 |
| AH3 | 17°22'/96°26' | 2904 | 22 | 13 | 0.943 (0.028) |
| AH4 | 17°27'/96°24' | 2530 | 13 | 10 | 0.949 (0.051) |
| AH46 | 18°58'/97°12' | 2910 | 20 | 9 | 0.916 (0.031) |
| AH47 | 19°31'/97°06' | 3090 | 14 | 12 | 0.978 (0.034) |
| mean | | | 17.25 | 11 | 0.95 |
| AF16 | 19°20'/101°21' | 2250 | 29 | 8 | 0.818 (0.043) |
| AF18 | 20°12'/104°43' | 2100 | 19 | 5 | 0.766 (0.049) |
| AF20 | 19°27'/103°56' | 2500 | 22 | 6 | 0.667 (0.092) |
| mean | | | 23.34 | 6.34 | 0.76 |
| AR22 | 19°35'/103°35' | 3330 | 23 | 10 | 0.889 (0.039) |
| AR48 | 19°31'/97°09' | 3510 | 16 | 11 | 0.950 (0.036) |
| AR49 | 19°41'/98°05' | 2760 | 18 | 9 | 0.889 (0.049) |
| AR55 | 19°23'/102°19' | 3030 | 17 | 8 | 0.823 (0.082) |
| mean | | | 18.50 | 9.50 | 0.89 |

Table 2 Chloroplast haplotype frequencies for *Abies guatemalensis*, *A. hickelí*, *A. flinckii* and *A. religiosa*, allele sizes at locus Pt 30204 and Pt 71936.

| | Relative frequency | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. guatemalensis* | | | | | | | | *A. hickelí* | | | | *A. flinckii* | | | *A. religiosa* | | |
| Haplotype | AG2 | AG7 | AG11 | AG41 | AG42 | AG44 | AG51 | AG52 | AH3 | AH4 | AH46 | AH47 | AF16 | AF18 | AF20 | AR22 | AR48 | AR49 | AR55 |
| 1. 123 142 | | | 0.091 | | | | | | | | | | | | | | | | |
| 2. 124 141 | | | 0.045 | | | | | | | | | | | | | | | | |
| 3. 124 142 | | 0.158 | 0.045 | | | 0.095 | | | | 0.077 | | 0.071 | | | | | 0.125 | | |
| 4. 124 143 | 0.08 | 0.053 | 0.409 | | | 0.048 | | | | | | 0.071 | | | | | | | |
| 5. 124 144 | | | 0.136 | | | | | | | | | 0.143 | | | | 0.043 | | | |
| 6. 124 145 | 0.08 | | 0.409 | | | | | | | | | | | | | | | | |
| 7. 124 146 | | | | | | | | | 0.091 | | | | | | | | | | |
| 8. 125 141 | 0.04 | | | | | 0.048 | | | | | | 0.071 | | | | | 0.062 | | |
| 9. 125 142 | 0.04 | 0.158 | | 0.059 | | 0.048 | 0.176 | 0.095 | | | 0.05 | 0.071 | | | | 0.043 | 0.188 | 0.278 | |
| 10. 125 143 | 0.16 | | 0.045 | | | 0.19 | 0.059 | 0.238 | 0.091 | 0.077 | 0.1 | 0.071 | | | | 0.261 | 0.125 | 0.167 | 0.059 |
| 11. 125 144 | 0.08 | 0.158 | 0.045 | | | | 0.647 | 0.048 | 0.045 | | | 0.143 | | | | 0.13 | | 0.056 | 0.118 |
| 12. 125 145 | 0.04 | 0.158 | | | | 0.048 | | 0.048 | | | | 0.071 | | | | 0.13 | | 0.056 | 0.059 |
| 13. 125 146 | | | | | | | | | 0.045 | | | 0.071 | | | | | | | |
| 14. 125 147 | | | | | | | | | | | | | | | | 0.043 | | | |
| 15. 126 141 | 0.04 | 0.053 | | | | | | | | | | | | | | | | 0.056 | |
| 16. 126 142 | 0.04 | | | 0.059 | 0.111 | 0.095 | | 0.048 | | 0.077 | 0.2 | | | | | | | | |
| 17. 126 143 | 0.2 | 0.053 | | 0.059 | 0.111 | 0.143 | | 0.095 | 0.045 | 0.077 | 0.15 | | | | | | 0.062 | 0.056 | 0.118 |
| 18. 126 144 | | 0.053 | 0.045 | | | | 0.118 | 0.143 | 0.182 | 0.077 | 0.1 | 0.071 | | | | | 0.062 | 0.167 | 0.412 |
| 19. 126 145 | | | 0.045 | | | | | | 0.091 | 0.077 | 0.1 | | | | | 0.174 | 0.062 | | 0.118 |
| 20. 126 146 | 0.04 | | | | | | | | 0.136 | 0.077 | 0.05 | | | | | 0.043 | | | |
| 21. 126 147 | | | | | | | | | | | | 0.071 | | | | | | | |
| 22. 126 148 | | | | | | | | | | | | | | | | 0.043 | | | |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23. 127 142 | 0.08 | | | | | 0.143 | | | | | 0.071 | | | | | 0.125 | | | |
| 24. 127 143 | 0.08 | 0.053 | 0.091 | 0.059 | 0.278 | 0.048 | 0.048 | 0.091 | 0.077 | 0.15 | | | | | | | 0.056 | 0.059 | |
| 25. 127 144 | | | | 0.059 | | 0.048 | | | 0.154 | | | | | | | | 0.111 | 0.059 | |
| 26. 127 145 | | 0.158 | | | | | 0.048 | 0.045 | 0.231 | | | | | | 0.087 | | | | |
| 27. 127 148 | | | | | | | | 0.045 | | | | | | | | | | | |
| 28. 128 142 | | | | 0.118 | | | | | | | | | | | | | | | |
| 29. 128 143 | | 0.053 | | 0.059 | 0.222 | | | | | 0.1 | | | | | | | | | |
| 30. 128 144 | | | | 0.118 | 0.222 | 0.048 | | | | | | | | | | | | | |
| 31. 128 145 | | | | 0.118 | | | | | | | | | | | | | | | |
| 32. 128 146 | | | | 0.059 | | | | | | | | | | | | 0.062 | | | |
| 33. 131 142 | | | | 0.059 | | | | | | | | | | | | | | | |
| 34. 131 143 | | | | 0.059 | 0.056 | | | | | | | | | | | | | | |
| 35. 131 144 | | | | | | | | | | | | | 0.053 | | | | | | |
| 36. 132 143 | | | | | | | | | | | | 0.034 | 0.263 | 0.091 | | | | | |
| 37. 132 144 | | | | | | | 0.048 | | | | | 0.034 | | 0.045 | | | | | |
| 38. 132 145 | | | | | | | | | | | | 0.103 | | | | | | | |
| 39. 133 143 | | | | | | | | | | | | 0.034 | | 0.045 | | | | | |
| 40. 133 144 | | | | 0.059 | | | | | | | | 0.276 | 0.316 | 0.545 | | 0.062 | | | |
| 41. 133 145 | | | | | | | | | | | | 0.31 | 0.316 | 0.227 | | | | | |
| 42. 133 146 | | | | | | | | | | | | 0.138 | | 0.045 | | | | | |
| 43. 134 145 | | | | | | | | | | | | | 0.053 | | | 0.062 | | | |
| 44. 134 146 | | | | | | | | 0.045 | | | | | | | | | | | |
| 45. 134 147 | | | | | | | | | | | | 0.069 | | | | | | | |
| 46. 143 124 | | 0.053 | | | | | | | | | | | | | | | | | |
| 47. 144 124 | | | | | | | 0.048 | | | | | | | | | | | | |
| 48. 145 124 | | | | | | | 0.048 | | | | | | | | | | | | |
| 49. 145 127 | | | | | | | 0.048 | | | | | | | | | | | | |
| 50. 146 127 | | | | 0.059 | | | | | | | | | | | | | | | |
| 51. 155 144 | | | | | | | | 0.045 | | | | | | | | | | | |

Table 3. Genetic differentiation among populations ($F_{ST}$ and $R_{ST}$) revealed by cpSSRs and gene flow in *A. guatemalensis*, *A. hickeli*, *A. flinckii* and *A. religiosa*.

| | $F_{ST}$ | *Nm* | $R_{ST}$ | *Nm* |
|---|---|---|---|---|
| *A. guatemalensis* | 0.131 (p= 0.000) | 3.32 | 0.104 (p= 0.003) | 4.32 |
| *A. hickeli* | 0.065 (p= 0.003) | 7.19 | 0.040 (p= 0.069) | 11.86 |
| *A. flinckii* | 0.029 (p= 0.118) | 16.74 | 0.116 (p= 0.012) | 3.81 |
| *A. religiosa* | 0.075 (p= 0.004) | 6.09 | 0.136 (p= 0.000) | 3.18 |

Table 4. Number of individuals analyzed (N), number of haplotypes ($N_{hapl}$), haplotype diversity ($H_e$), number of polymorphic loci studied ($N_p$) and total genetic differentiation among populations ($F_{ST}$ or $R_{ST}$) revealed by cpSSRs for different species of *Abies*, *Pinus* and *Pseudotsuga menziesii*.

| Species | N | $N_{hapl}$ | $N_p$ | $H_e$ | $F_{ST}/R_{ST}$ | Reference |
|---|---|---|---|---|---|---|
| *A. alba* | 714 | 90 | 2 | 0.85 | $F_{ST} = 0.133$ | Vendramin et al. 1999 |
| *A. fraseri* | 26 | 16 | 2 | 0.84 | - | Clark et al. 2000 |
| *A. balsamea* | 26 | 20 | 2 | 0.95 | - | Clark et al. 2000 |
| *A. guatemalensis* | 160 | 31 | 2 | 0.87 | $F_{ST} = 0.131$<br>$R_{ST} = 0.104$ | This study |
| *A. religiosa* | 74 | 21 | 2 | 0.89 | $F_{ST} = 0.075$<br>$R_{ST} = 0.136$ | This study |
| *A. flinckii* | 70 | 10 | 2 | 0.76 | $F_{ST} = 0.029$<br>$R_{ST} = 0.116$ | This study |
| *A. hickeli* | 69 | 21 | 2 | 0.95 | $F_{ST} = 0.065$<br>$R_{ST} = 0.040$ | This study |
| *P. halepensis* | 127 | 28 | 8 | 0.222 | $F_{ST} = 0.308$<br>$R_{ST} = 0.212$ | Bucci et al. 1998 |
| *P. resinosa* | 159 | 23 | 9 | 0.568 | $F_{ST} = 0.121$ | Echt et al. 1998 |
| *P sylvestris* | 330 | 174 | 13 | 0.978 | $R_{ST} = 0.32$ | Provan et al. 1998 |
| *P. pinaster* | 240 | 34 | 7 | 0.81 | $R_{ST} = 0.235$ | Vendramin et al, 1998 |
| *P. nelsonii* | 256 | 27 | 4 | 0.531 | $F_{ST} = 0.131$<br>$R_{ST} = 0.05$ | Cuenca 2001 |
| *P. pinceana* | 154 | 14 | 4 | 0.379 | $F_{ST} = 0.785$<br>$R_{ST} = 0.938$ | Escalante 2001 |
| *Pseudotsuga menziesii* | 323 | 5 | 3 | 0.38 | $F_{ST} = 0.02$ | Viard et al. 2001 |

Table 5. Linkage Disequilibrium between two microsatellite loci for each haplotype, obtained with: r2=(PA-PAPB)2/PA(1-PA)PB(1-PB) (Hill & Robertson 1968)

| Haplotype | Population | r2 |
|---|---|---|
| 143 125 | AG2 | 0.012865028 |
| | AH3 | 0.094501353 |
| | AH4 | 0.277717588 |
| | AG11 | 0.001029338 |
| | AR22 | 0.226754301 |
| | AG44 | 0.040848016 |
| | AH46 | 0.019607843 |
| | AH47 | 0.023118814 |
| | AR48 | 0.083017154 |
| | AR49 | 0.003119193 |
| | AG51 | 0.008388336 |
| | AG52 | 0.096205061 |
| | AR55 | 0.000440936 |
| Mean | | 0.06827792 |
| 145 124 | AG2 | 0.26046176 |
| 143 124 | AG2 | 0.000268286 |
| | AG7 | 0.002594002 |
| | AG11 | 0.067783555 |
| | AG44 | 0.00593413 |
| | AH47 | 0.104618658 |
| Mean | | 0.036239726 |
| 144 125 | AG2 | 0.154589372 |
| | AH3 | 0.000743161 |
| | AG7 | 0.23177533 |
| | AG11 | 0.04082607 |
| | AR22 | 0.016878303 |
| | AH47 | 0.021960262 |
| | AR49 | 0.304191827 |
| | AG51 | 0.041097921 |
| | AG52 | 0.065887239 |
| | AR55 | 0.009350579 |
| Mean | | 0.088730006 |
| 143 126 | AG2 | 0.020786199 |
| | AH3 | 0.078072907 |
| | AH4 | 0.003386653 |
| | AG7 | 0.017455277 |
| | AG41 | 0.052260778 |
| | AG42 | 0.035829129 |
| | AG44 | 0.037651199 |
| | AH46 | 0.375 |
| | AR48 | 0.030490335 |
| | AR49 | 0.011244561 |
| | AG52 | 0.004050086 |
| | AR55 | 0.028229188 |
| Mean | | 0.057871359 |
| 143 127 | AG2 | 0.00030525 |

| | | |
|---|---|---|
| | AH3 | 0.094501353 |
| | AH4 | 0.020007531 |
| | AG7 | 0.002594002 |
| | AG11 | 0.083578083 |
| | AG41 | 0.052260778 |
| | AG42 | 0.192232139 |
| | AG44 | 0.065887239 |
| | AH46 | 0.176470588 |
| | AR49 | 0.003282792 |
| | AG52 | 0.006872966 |
| | AR55 | 0.052260778 |
| Mean | | 0.062521125 |
| 141 126 | AG2 | 0.01294757 |
| | AG7 | 0.298250304 |
| | AR49 | 0.154066577 |
| Mean | | 0.155088151 |
| 142 126 | AG2 | 0.004289216 |
| | AH4 | 0.010167407 |
| | AG41 | 0.027352425 |
| | AG42 | 0.437570304 |
| | AG44 | 0.000436739 |
| | AH46 | 0.055555556 |
| | AG52 | 0.002015488 |
| Mean | | 0.07676959 |
| 142 127 | AG2 | 0.1638322 |
| | AG44 | 0.064006064 |
| | AH47 | 0.280705814 |
| | AR48 | 0.183300718 |
| Mean | | 0.172961199 |
| 146 126 | AG2 | 0.088541667 |
| | AH3 | 0.001404172 |
| | AH4 | 0.133261105 |
| | AR22 | 0.127221482 |
| | AH46 | 0.035087719 |
| Mean | | 0.077103229 |
| 145 125 | AG2 | 0.000420875 |
| | AR22 | 0.206165476 |
| | AG44 | 0.100991748 |
| | AH47 | 0.076426265 |
| | AR49 | 0.047372272 |
| | AG52 | 0.002492362 |
| | AR55 | 0.011935153 |
| Mean | | 0.063686307 |
| 141 125 | AG2 | 0.007397343 |
| | AG44 | 0.100991748 |
| | AH47 | 0.076426265 |
| | AR48 | 0.110163468 |
| Mean | | 0.073744706 |

| | | |
|---|---|---|
| 142 125 | AG2 | 0.010003307 |
| | AG7 | 0.072364146 |
| | AR22 | 0.028848018 |
| | AG41 | 0.15056352 |
| | AG44 | 0.118760229 |
| | AH46 | 0.006535948 |
| | AH47 | 0.030819719 |
| | AR48 | 0.009776998 |
| | AR49 | 0.307479224 |
| | AG51 | 0.028575832 |
| | AG52 | 0.0377257 |
| Mean | | 0.072859331 |
| 144 155 | AH3 | 0.125481848 |
| 144 126 | AH3 | 0.067846161 |
| | AH4 | 0.003386653 |
| | AG7 | 0.017455277 |
| | AG11 | 0.04082607 |
| | AH46 | 0.074074074 |
| | AH47 | 0.014146409 |
| | AR48 | 0.088774762 |
| | AR49 | 0.124250257 |
| | AG51 | 0.041097921 |
| | AG52 | 0.15161371 |
| | AG55 | 0.018006551 |
| Mean | | 0.058316168 |
| 145 126 | AH3 | 0.029101957 |
| | AH4 | 0.034259129 |
| | AG11 | 0.470686382 |
| | AR22 | 0.112712097 |
| | AH46 | 0.074074074 |
| | AR48 | 0.088774762 |
| | AR55 | 0.000514471 |
| Mean | | 0.115731839 |
| 146 124 | AH3 | 0.214701344 |
| 145 127 | AH3 | 0.023436185 |
| | AH4 | 0.148526979 |
| | AG7 | 0.70168072 |
| | AR22 | 0.148418832 |
| | AG52 | 0.205507596 |
| Mean | | 0.245514062 |
| 148 127 | AH3 | 0.211782981 |
| 146 125 | AH3 | 0.005134826 |
| | AH47 | 0.076426265 |
| Mean | | 0.040780546 |
| 146 134 | AH3 | 0.101056999 |
| 144 127 | AH4 | 0.050623903 |

| | | |
|---|---|---|
| | AG41 | 0.052260778 |
| | AG44 | 0.041344837 |
| | AR49 | 0.099292286 |
| | AR55 | 0.004276655 |
| Mean | | 0.049559692 |
| 142 124 | AH4 | 0.458288191 |
| | AG7 | 0.23177533 |
| | AG11 | 0.063299652 |
| | AG44 | 0.056799182 |
| | AH47 | 0.002793811 |
| | AR48 | 0.183300718 |
| Mean | | 0.166042814 |
| 143 128 | AG7 | 0.209276488 |
| | AG41 | 0.059638013 |
| | AG42 | 0.100270009 |
| | AH46 | 0.267143013 |
| Mean | | 0.159081881 |
| 124 143 | AG7 | 1 |
| 141 124 | AG11 | 0.02696829 |
| 144 124 | AG11 | 0.001725419 |
| | AR22 | 0.213298262 |
| | AH47 | 0.035682946 |
| Mean | | 0.083568876 |
| 142 123 | AG11 | 0.635993011 |
| 144 133 | AF16 | 0.042357723 |
| | AF18 | 0.128662571 |
| | AF20 | 0.041605164 |
| | AG41 | 0.204106089 |
| | AR48 | 0.462686567 |
| Mean | | 0.175883623 |
| 147 134 | AF16 | 1 |
| 145 132 | AF16 | 0.029253576 |
| 145 133 | AF16 | 0.000402441 |
| | AF18 | 0.128662571 |
| | AF20 | 0.046224426 |
| Mean | | 0.058429813 |
| 143 132 | AF16 | 0.053540666 |
| | AF18 | 1 |
| | AF20 | 0.380731344 |
| Mean | | 0.47809067 |
| 146 133 | AF16 | 0.050833158 |
| | AF20 | 0.007417103 |

| | | |
|---|---|---|
| Mean | | 0.02912513 |
| 144 132 | AF16 | 0.012253063 |
| | AF20 | 0.044060966 |
| | AG52 | 0.161429278 |
| Mean | | 0.072581102 |
| 143 133 | AF16 | 0.028721559 |
| | AF20 | 0.380731344 |
| Mean | | 0.204726451 |
| 144 131 | AF18 | 0.096115881 |
| 145 134 | AF18 | 0.096115881 |
| | AR48 | 0.462686567 |
| Mean | | 0.279401224 |
| 147 125 | AR22 | 0.028848018 |
| 148 126 | AR22 | 0.127221482 |
| 142 128 | AG41 | 0.008105444 |
| 142 131 | AG41 | 0.027352425 |
| 127 146 | AG41 | 1 |
| 144 128 | AG41 | 0.001194601 |
| | AG42 | 0.357326478 |
| | AG44 | 0.480318443 |
| Mean | | 0.279613174 |
| 145 128 | AG41 | 0.150261662 |
| 143 131 | AG41 | 0.052260778 |
| | AG42 | 0.029616548 |
| Mean | | 0.040938663 |
| 146 128 | AG41 | 0.070420184 |
| | AR48 | 1 |
| Mean | | 0.53521009 |
| 147 129 | AH47 | 0.458023139 |
| 127 145 | AG52 | 0.480318443 |
| 124 146 | AG52 | 0.205507596 |

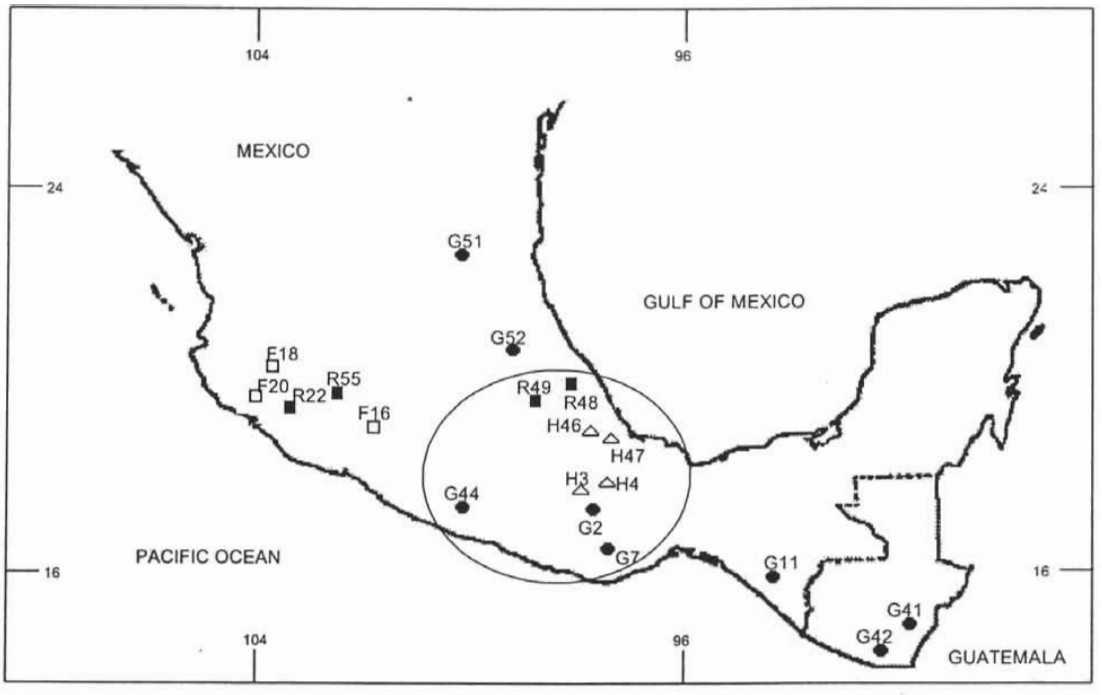
**Figure Captions**

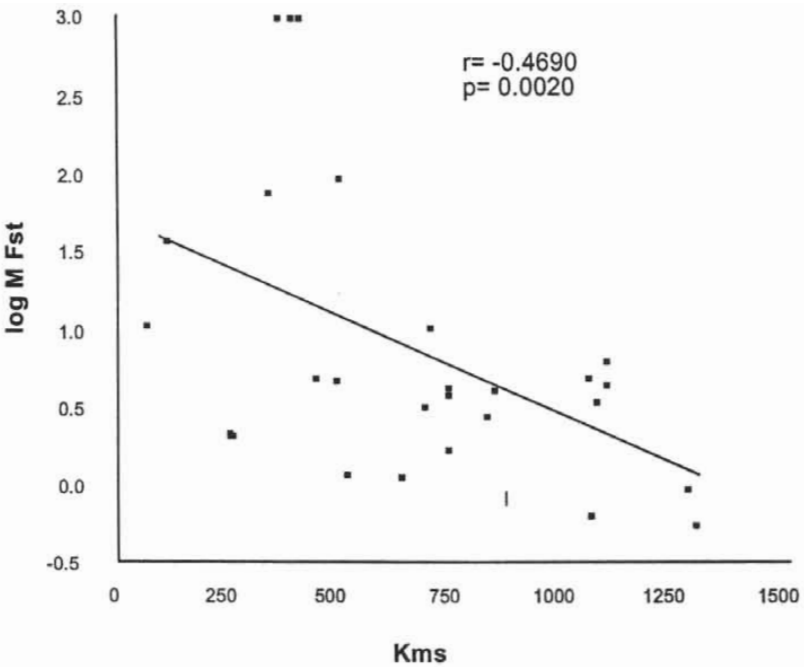
**Fig 1.** Locations of populations of *Abies guatemalensis* (G), *A. religiosa* (R), *A. hickeli* (H) and *A.flinckii* (F) sampled for cpSSRs analysis.

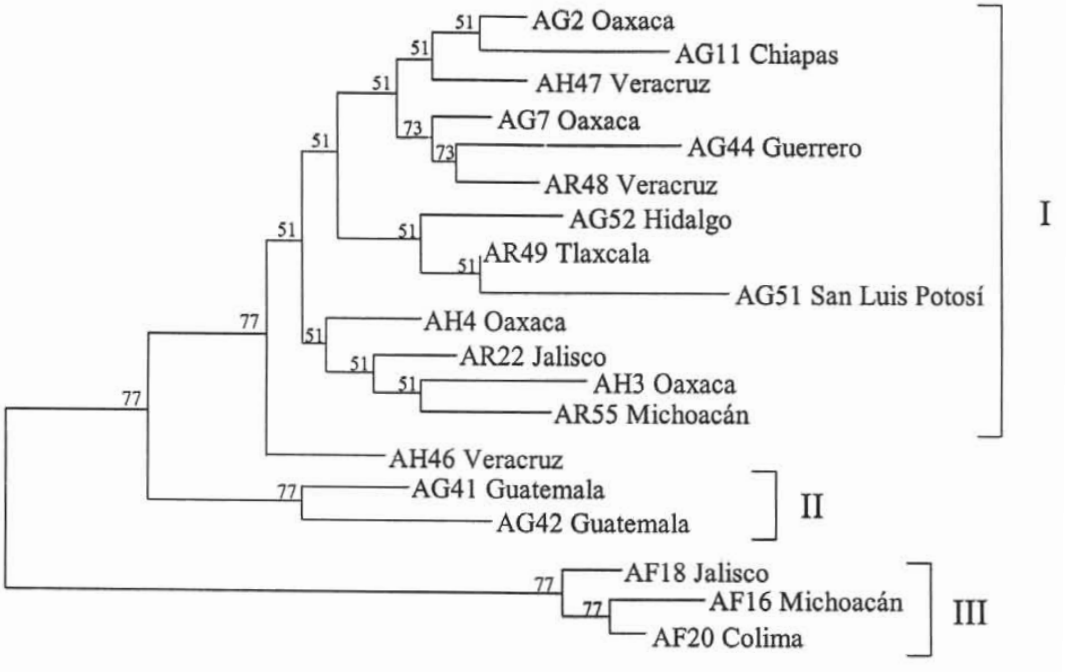
**Fig 2.** Plot of pairwise $\log_{10}M$ ($M$ = gene flow calculated from $F_{ST}$) values, against $\log_{10}k$ ($k$ = geographic distance between pairs of populations), for *A. guatemalensis*.

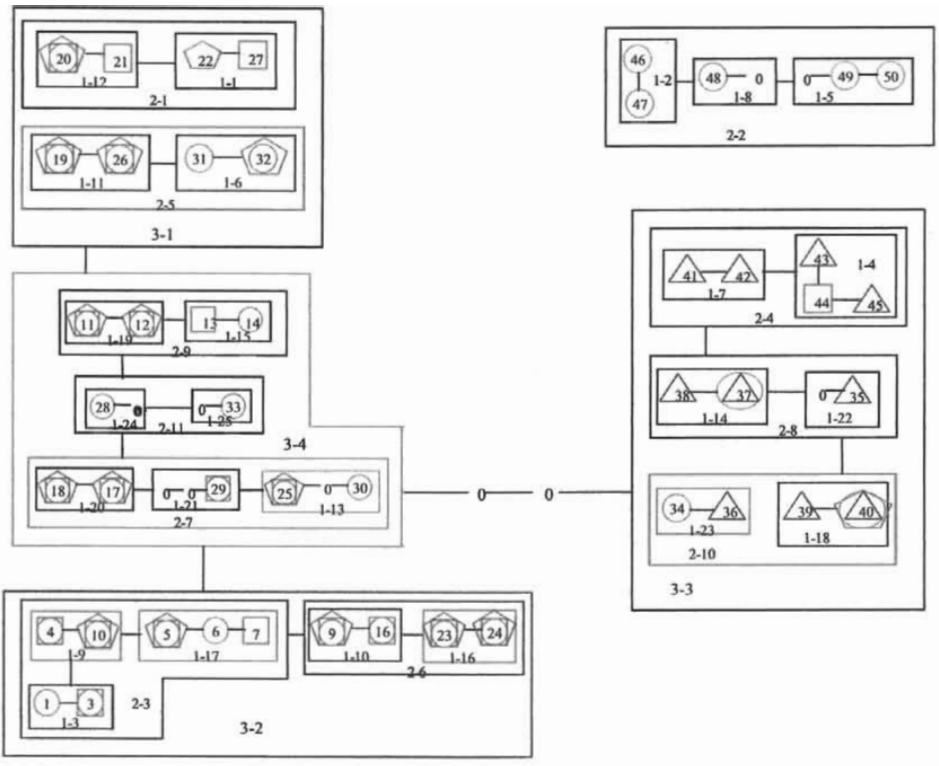
**Fig 3.** Neighbor-joining phenogram based on Nei's unbiased genetic distances between populations of *Abies guatemalensis* (G), *A. religiosa* (R), *A. hickeli* (H) and *A.flinckii* (F). Number are bootstrap values (in percentages), values below 50 are not shown.

**Fig 4.** Nested unrooted haplotype network. Haplotypes are designated by numbers, frequency of each haplotype is shown in table 2. Haplotypes pertain to taxa as follows: *A. guatemalensis* ◯ *A. hickeli* □ *A. religiosa* ⬠ and *A.. flinckii* △ Lines separate haplotypes or clades that differ by one mutational step. A "0" designates inferred intermediate haplotypes. Clades outlined in color show significant clade-geographical associations. Outlined in red are clades where isolation by distance was detected, in blue clade 1-17 where a fragmentation event was detected and in green clade 1-13, where a range expansion event was detected. Haplotypes 2, 8, 15 and 51 are not included in the network, because of limitations in number of haplotypes accepted in the program.


104
96
MEXICO
24
24
G51
GULF OF MEXICO
G52
E18
F20
R22
R55
F16
R49
R48
H46
H47
H3
H4
G44
G2
G7
PACIFIC OCEAN
16
16
G11
G41
G42
GUATEMALA
104
96


r= -0.4690
p= 0.0020
log M Fst
Kms
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
250
500
750
1000
1250
1500


AG2 Oaxaca
AG11 Chiapas
AH47 Veracruz
AG7 Oaxaca
AG44 Guerrero
AR48 Veracruz
AG52 Hidalgo
AR49 Tlaxcala
AG51 San Luis Potosí
AH4 Oaxaca
AR22 Jalisco
AH3 Oaxaca
AR55 Michoacán
AH46 Veracruz
AG41 Guatemala
AG42 Guatemala
AF18 Jalisco
AF16 Michoacán
AF20 Colima
I
II
III


20
21
1-12
22
27
1-1
2-1
19
26
1-11
31
32
1-6
2-5
3-1
46
47
1-2
48
0
1-8
0
49
50
1-5
2-2
41
42
1-7
43
1-4
44
45
2-4
38
37
1-14
0
35
1-22
2-8
34
36
1-23
39
40
1-18
2-10
3-3
11
12
1-19
13
14
1-15
2-9
28
0
1-24
2-11
0
33
1-25
3-4
18
17
1-20
0
0
29
1-21
2-7
25
0
30
1-13
0
0
4
10
1-9
5
6
7
1-17
9
16
1-10
23
24
1-16
2-6
1
3
1-3
2-3
3-2

## DISCUSIÓN GENERAL Y CONCLUSIONES

La estructura genética de las especies del género *Abies* distribuidas al sur de la República Mexicana y en Guatemala, *Abies guatemalensis, A. religiosa, A. hickeli y A. flinckii,* presenta procesos como deriva génica, endogamia y bajos niveles de flujo génico a nivel isoenzimático. Por otro lado, la distribución geográfica de los haplotipos del cloroplasto y los datos de isoenzimas sugieren una diversificación relativamente reciente de estas especies.

La destrucción y fragmentación del hábitat han restringido la distribución de muchas especies de plantas a poblaciones pequeñas y aisladas, como es el caso para algunas poblaciones de *Abies* estudiadas. Estas poblaciones son más susceptibles a la extinción porque son propensas a la pérdida de variación genética por deriva génica y endogamia (Frankham 1995, Keller y Waller 2002). La extinción de una especie se traduce en una pérdida de diversidad a nivel familia a la cual le pertenece la especie. Esta pérdida de diversidad es mayor cuando la especie extinta tiene poca relación con las especies sobrevivientes de la familia, que cuando el parentesco es más cercano (Caballero y Toro, 2002).

Cuando las poblaciones de una especie permanecen pequeñas y aisladas durante varias generaciones, pueden ocurrir dos cosas desde el punto de vista genético. Por un lado, es posible que por deriva génica se fijen o pierdan distintos alelos al azar y los niveles de variación genética cuantitativa necesaria para la evolución adaptativa disminuyen (Keller y Waller 2002), y por lo tanto, la capacidad de las poblaciones para adaptarse a distintas condiciones. Por otro lado, es posible que se acumulen mutaciones deletéreas, ya que la selección es menos efectiva en poblaciones pequeñas (Lynch et al. 1995). Estos dos procesos son graduales, pero la endogamia actúa de una manera más rápida, incrementando la frecuencia de individuos homócigos para alelos idénticos por descendencia, lo que resulta en una reducción en la adecuación a la cual se le ha llamado depresión por endogamia (Ellstrand y Elam 1993, Keller y Waller 2002).

**Procesos evolutivos en *Abies guatemalensis*, *A. hickelí*, *A. religiosa* y *A. flinckii* que se infieren a partir de dos tipos de marcadores genéticos.**

En el material genético de cada especie tenemos información acerca de su historia evolutiva y, al analizar los patrones de distribución de esta variación genética y su genealogía es posible inferir procesos evolutivos que ocurren en conjuntos de especies relacionadas. Existen distintos tipos de marcadores genéticos, que por su tasa de mutación pueden ser más o menos variables, lo cual permite analizar procesos evolutivos a distintas escalas. De la misma manera, la herencia de los marcadores puede ser distinta. En este estudio se utilizaron marcadores que tienen una herencia nuclear, isoenzimas y microsatélites de cloroplasto, estos últimos son regiones muy variables y en el caso de *Abies*, tienen una herencia paterna (Wagner 1992). La comparación de los patrones de variación entre marcadores de herencia nuclear y paterna permiten hacer inferencias acerca del movimiento del polen (McCauley 1995). Por otro lado, ambos tipos de marcadores pueden estar aportando información sobre distintos procesos de la historia de estas especies. Por su tasa de mutación más baja, las isoenzimas podrían dar información sobre la historia más antigua de las especies: se encontraron niveles relativamente bajos de diversidad genética, deficiencia de heterócigos y altos niveles de diferenciación entre poblaciones dentro de especies, lo que sugiere la ocurrencia de deriva génica, endogamia y bajos niveles de flujo génico. En el análisis de microsatélites de cloroplasto (cpSSRs) se encontraron niveles relativamente altos de flujo génico y niveles altos de diversidad. El cloroplasto tiene una herencia paterna en *Abies* (Wagner 1992) y por lo tanto puede migrar por polen y por semilla. Bajo cualquier modelo, ya sea el modelo de islas o el de aislamiento por distancia, se esperaría una mayor diferenciación para genes de herencia paterna en comparación con genes de herencia nuclear (Ennos 1994). En el caso de las especies de *Abies* estudiadas se encontró una mayor diferenciación con isoenzimas. Los bajos niveles de diferenciación encontrados con cpSSRs y los niveles relativamente más altos encontrados con isoenzimas

(genes nucleares) entre poblaciones de *Abies* en el sur de México y Guatemala, pueden ser atribuidos a una dispersión limitada de semillas y mayor movimiento de polen. De la misma manera, se infiere que el aislamiento por distancia está promoviendo cierto grado de diferenciación. Aunque no se tienen datos empíricos acerca de la dispersión del polen particularmente en *Abies*, Liepelt et al. (2002) demostraron que el flujo génico por polen es mucho mayor que el que previamente sugerían los datos de isoenzimas para *Abies alba*.

Sin embargo, los estimados de flujo génico deben de tomarse con cuidado en estas especies, ya que las poblaciones son relativamente jóvenes y las poblaciones que estuvieron históricamente en contacto y que actualmente están aisladas pueden tener eventualmente distintos alelos fijos en el equilibrio, pero el tiempo requerido para alcanzar este equilibrio puede ser mayor que la edad de la mayoría de estas especies (Neigel 2002).

En lo que respecta a las relaciones entre las especies, el fenograma realizado con los datos de isoenzimas sugiere la existencia de tres grupos principales: 1) *A. guatemalensis,* que incluye a *A. flinckii* (formando un grupo monofilético), 2) *A. hickeli* y 3) *A. religiosa*. Las ramas que separan los tres grupos son bastante cortas, lo que es consistente con la poca diferenciación encontrada entre especies y puede estar sugiriendo una separación reciente de las especies o flujo génico actual o reciente entre lo grupos.

El análisis de microsatélites de cloroplasto (cpSSRs) también sugiere que las especies de *Abies* del sur se separaron recientemente, y aunque probablemente se encuentren aisladas reproductivamente en la actualidad, divergieron tan recientemente que algunas poblaciones pueden estar todavía en proceso de especiación y todavía comparten muchos polimorfismos. Estos análisis indican que hay tres grupos genéticos bien diferenciados: 1) *A. flinckii*, que ha tenido una mayor divergencia, 2) otro grupo que incluye a poblaciones de *A. guatemalensis* de Guatemala y 3) un grupo que contiene a poblaciones de *A. hickeli*, *A. religiosa* y *A. guatemalensis* en México, en el cual encontramos

haplotipos ancestrales. Los resultados también sugieren que las poblaciones en la parte central de la distribución son las poblaciones ancestrales y que probablemente a partir de éstas migraron individuos hacia otras regiones.

Con ambos marcadores se puede diferenciar a *A. flinckil.* Sin embargo el estudio de isoenzimas pudo separar a los taxa *A. guatemalensis*, *A. religiosa* y *A. hickeli*, con excepción de algunas poblaciones (G44, G52 y H46), las cuales están agrupadas con *A. religiosa* y se encuentran aisladas de sus poblaciones conespecíficas y cercanas a poblaciones de *A. religiosa*, lo cual puede explicar estas diferencias. En el caso de los microsatélites, es posible separar a *A. flinckii*, como se mencionó anteriormente, y a las poblaciones de *A. guatemalensis* pertenecientes a Guatemala.

Se ha propuesto que el género *Abies*, al igual que otras especies de climas templados, migraron hacia el sur, hasta México, durante los periodos de glaciación en el Pleistoceno, que abarca desde hace 1.6 millones de años, hasta hace 11,000 años, habiendo ocurrido el último máximo glacial hace 18,000 años (Deevey, 1949; Dressler 1954 en Perry et al. 1998). En contraste, otros autores han postulado que estos taxa empezaron a migrar hacia México y América Central a principios del Terciario (hace 55 millones de años) y durante el Terciario medio (hace 23 millones de años) (Chaney 1936; Steyermark 1950; Braun 1955; Martin y Harrel 1957 en Perry et al. 1998). El primer registro fósil que se tiene en México del género *Abies* data de mediados del Plioceno (hace cinco millones de años) y es de la formación del Paraje Solo en Veracruz (Graham, 1993, 1999), por lo que se considera que efectivamente, la divergencia de estas especies puede ser un evento relativamente reciente en una escala evolutiva. Estas poblaciones pueden no haber estado aisladas durante mucho tiempo desde que experimentaron una disminución en su rango y una fragmentación en sus poblaciones en el periodo que siguió a la última glaciación (~18 000 años), durante el periodo de calentamiento que tuvo su máximo hace ~6 000 años (Aguirre-Planter et al. 2000) y considerando que las especies de *Abies* tienen tiempos generacionales relativamente largos. Si tomamos en cuenta que *Abies* tiene un tiempo generacional de aproximadamente 33 años

(Silvertown y Dodd 1999) y que han estado separadas por ~9 000 años, entonces han pasado alrededor de 272 generaciones, a lo más, desde que las poblaciones se encuentran aisladas.

La combinación de diferentes tipos de datos o diferentes marcadores con distintas tasas de mutación permite distinguir entre fenómenos recientes e históricos y pueden aportar un panorama más amplio de la historia de estas especies. Estos métodos, al mismo tiempo, constituyen herramientas importantes para tomar decisiones sobre conservación y manejo de las poblaciones y de las especies. Desde el punto de vista de la genética de la conservación, el análisis de isoenzimas, que muestra niveles altos de diferenciación entre poblaciones, sugiere la importancia de preservar la mayor cantidad posible de poblaciones genéticamente distintas para conservar la mayor proporción posible de la variación genética de las especies. Por otro lado, los resultados obtenidos con cpSSR sugieren que se necesitan preservar poblaciones de tres grupos genéticos distintos y poblaciones que tienen haplotipos únicos, como G41, G52, G7, R22 y H3, así como también poner especial atención en preservar las poblaciones de *A. guatemalensis*, una especie amenazada (FAO 1986; CITES, Appendix 1, http://www.cites.org/), que es la que posee los niveles más altos de diferenciación entre sus poblaciones.

El propósito general en biología de la conservación es el de proteger la diversidad biológica y los procesos que la mantienen (Moritz 2002). Originalmente, la identificación de unidades de conservación intraespecíficas, es decir una población o grupo de poblaciones que amerita una prioridad de conservación, se ha basado en el reconocimiento de grupos monofiléticos (Moritz 1994). Dentro de un árbol filogenético cada rama o grupo de ramas monofilética se consideraría una unidad evolutiva significativa (ESU). Sin embargo, la manera de definir ESUs ha generado discusiones en la literatura, y se han hecho distintas críticas a esta definición (Crandall et al. 2000, Moritz 2002, Moran 2002).

Crandall et al. (2000) proponen que lo importante es mantener la estructura poblacional histórica, es decir, es importante considerar los procesos

evolutivos. Por lo tanto, y con este fin, el uso de redes de poblaciones es más útil cuando se tienen datos de genética de poblaciones. Para preservar procesos evolutivos el objetivo del manejo y la conservación debería ser el de preservar la red natural de conexiones genéticas entre poblaciones, más que sólo distintas poblaciones aisladas dentro de la red. Esto asegura que los procesos que mantienen la diversidad adaptativa y el potencial evolutivo sean conservados (Crandall et al. 2000). En este contexto, aparentemente la única especie que se encuentra bien diferenciada es *A. flinckii* y es la única que está funcionando como una entidad evolutiva independiente, las otras especies se encuentran conectadas genéticamente.

**Perspectivas**

Se han propuesto distintas clasificaciones basadas en características morfológicas para las especies de *Abies* estudiadas; sin embargo, para algunos taxa no existe consenso entre los autores (Martínez 1948, Liu 1971, Rushforth 1989, Farjon 1990, 1998), lo que sugiere que la taxonomía con información morfológica no ha podido resolver de manera adecuada la clasificación de estas especies. De la misma manera, en este estudio se pudo comprobar que la clasificación de estas especies no es muy clara y no es posible identificar a todas las especies genéticamente. Por lo tanto, es necesario encontrar otros marcadores moleculares para tratar de diferenciar a las especies. Algunas secuencias podrían ser utilizadas con este fin. Por ejemplo, las regiones secuenciadas por Suyama et al. (2000), quienes secuenciaron ocho regiones del cloroplasto en total: *rbc*L, *mat*K, y seis regiones espaciadoras, para poder clarificar la posición filogenética de cinco especies de *Abies* de Japón. Por otro lado, Gernandt et al. (2003) secuenciaron ADN de cloroplasto de regiones de *mat*K, *rbc*L y *rpl*16 para inferir las relaciones dentro de los pinos piñoneros (*Pinus* subsecciones *Cembriodes* y *Nelsoniae*). Es necesario poder clarificar las relaciones entre las especies de *Abies* de México, para poder entender y explicar de una manera mas detallada y contundente lo encontrado en este trabajo.

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