HIGH GENETIC DIVERSITY WITHIN BUT LIMITED DIFFERENTIATION AMONG POPULATIONS OF THE VULNERABLE GUATEMALAN FIR

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RASMUSSEN KK, STRANDBY U & KOLLMANN J. 2010. High genetic diversity within but limited differentiation among populations of the vulnerable Guatemalan fir. Genetic diversity in fragmented tree populations may reflect provenance variation and the relationship between genetic diversity and population characteristics should be known to improve conservation strategies. We chose the vulnerable *Abies religiosa* subsp. *mexicana* (syn. *Abies guatemalensis*) to study genetic diversity within and among 18 populations in Guatemala (284 trees, seven microsatellite markers). Genetic diversity was high in all populations (observed heterozygosity = 0.70–0.90), while six populations deviated from Hardy–Weinberg equilibrium. Inbreeding within populations (F_{is}) was significant in five populations, among them two peripheral populations. A cluster analysis and a principal component analysis (PCA) indicated existence of one large metapopulation with the two peripheral populations as outliers. Longitude, geographic isolation and expected heterozygosity were correlated with the first PCA axis, while altitude was correlated with the second axis. Mountain range, forest size and fitness traits of the provenances were not correlated with genetic diversity. The study indicated that peripheral populations of *A. religiosa* subsp. *mexicana* contained unique genetic information which should be included in *ex situ* conservation programmes, whereas the other populations in Guatemala showed little evidence for inbreeding or genetic erosion. We conclude that fragmented conifer populations can maintain high genetic diversity, while differentiation among populations can be weak.

Keywords: *Abies religiosa* subsp. *mexicana* (syn. *Abies guatemalensis*), fragmentation, gene flow, inbreeding, microsatellite marker, peripheral population

RASMUSSEN KK, STRANDBY U & KOLLMANN J. 2010. Kepelbagaian yang tinggi di dalam populasi fir yang terancam di Guatemala tetapi pembezaan terhad di kalangannya. Kepelbagaian genetik dalam populasi pokok yang berpecah-pecah mungkin menunjukkan variasi provenans. Hubungan antara kepelbagaian genetik dengan ciri populasi perlu diketahui untuk menambah baik strategi pemuliharaan. Kami memilih *Abies religiosa* subsp. *mexicana* (sinonim *Abies guatemalensis*) yang terancam untuk mengkaji kepelbagaian genetik di dalam dan di kalangan 18 populasi di Guatemala (248 pokok, tujuh penanda mikrosatelit). Kepelbagaian genetik adalah tinggi dalam semua populasi (heterozigositi cerapan = 0.70–0.90) tetapi enam populasi tersisih daripada keseimbangan Hardy–Weinberg. Di dalam populasi, pembiakbakaan dalam (F_{is}) adalah signifikan bagi lima populasi dan di kalangan populasi pula, nilainya signifikan bagi dua populasi pinggiran. Analisis kelompok serta analisis komponen prinsipal (PCA) menunjukkan kewujudan satu metapopulasi yang besar dan dua populasi pinggiran tersebut sebagai pencilan. Longitud, pemencilan geografi dan heterozigositi terjangka berkorelasi dengan paksi PCA yang pertama sementara altitud berkorelasi dengan paksi yang kedua. Banjaran gunung, saiz hutan dan ciri kebugaran provenans tidak berkorelasi dengan kepelbagaian genetik. Kajian ini menunjukkan bahawa populasi pinggiran *A. religiosa* subsp. *mexicana* mengandungi maklumat genetik unik yang harus diambil kira dalam program pemuliharaan *ex situ*. Sebaliknya populasi lain di Guatemala menunjukkan hanya sedikit bukti berlakunya pembiakbakaan dalam atau hakisan genetik. Kami membuat kesimpulan bahawa populasi konifer yang berpecah-pecah dapat mengekalkan kepelbagaian genetik yang tinggi tetapi pembezaan di kalangan populasi mungkin lemah.

INTRODUCTION

Population genetics has become an essential tool to support conservation and management of endangered plant species (Newton *et al.* 1999, Allendorf & Luikart 2007). Numerous studies have shown reduced gene flow and lower genetic diversity in small and isolated populations, especially in recently fragmented landscapes (e.g. Oostermeijer

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et al. 2002, Galeuchet *et al.* 2005, Hensen & Oberprieler *et al.* 2005). In some of these landscapes, destruction of habitats by humans has removed the natural ability of the species to adapt to changing site conditions and has led to genetic erosion (for a review see Willi *et al.* 2006). Low genetic diversity in fragmented populations is often correlated with reduced fitness. Thus, genetic diversity is a useful measure of population viability (Ellstrand & Elam 1993, Fischer & Matthies 1998, Fischer *et al.* 2003).

At the margins of a distribution range, plant populations tend to be naturally fragmented because suitable habitats are only available in specific and more restricted locations (Gaston 2003, Travis & Dytham 2004), which can be remnants of former wider distributions (Premoli *et al.* 2007). Within-population genetic diversity is often lower and among-population differentiation is higher towards range margins (Eckert *et al.* 2008). Moreover, peripheral populations may contain genotypes which could become important for future evolution in response to changing habitat conditions (Eckstein *et al.* 2006, Cornman & Arnold 2007). Such populations are particularly endangered by changes in landuse and climate (Oostermeijer *et al.* 2002, Jacquemyn *et al.* 2007, Rasmussen & Kollmann 2008). Range margins should be included when discussing the genetic structure of endangered plant species and peripheral populations are particularly interesting for analysing genetic variation within and among populations.

In addition to potential effects of population size and range margin, genetic differentiation among populations depends on the breeding system (Hamrick *et al.* 1992), a life history trait which may also affect the genetic response of plant species to habitat fragmentation (Honnay & Jacquemyn 2007). Many conifer species have relatively high levels of overall genetic diversity and little differentiation among populations. This is caused largely by longevity of the individuals and extensive gene flow due to wind pollination (Terrab *et al.* 2007); for contrasting examples see Premoli *et al.* (2007).

Guatemalan fir, *Abies religiosa* subsp. *mexicana* (syn. *Abies guatemalensis*), is endemic to the highlands of Mexico and Guatemala (Donahue *et al.* 1985, Jaramillo-Correa *et al.* 2008, Strandby *et al.* 2009). It has its centre of distribution in eastern Mexico and western Guatemala and the present range margin is in eastern Guatemala (Andersen *et al.* 2006). Recent deforestation rates have been particularly high in this subtropical region (Echeverría *et al.* 2007) with negative effects on biodiversity (Rey-Benayas *et al.* 2007). Distribution, regeneration and socio-economic aspects of *A. religiosa* subsp. *mexicana* are well studied (Andersen *et al.* 2008a, b, Kollmann *et al.* 2008, Strandby & Olsen 2008), but, except for the publications by Aguirre-Planter *et al.* (2000) and Jaramillo-Correa *et al.* (2008), little is known about population genetics which is important for conservation management. Given the current threats to this conifer it is urgent to identify the potential for *in situ* conservation areas and to develop a background for collecting germplasm of specific provenances (cf. Andersen *et al.* 2008b).

For these reasons, *A. religiosa* subsp. *mexicana* was chosen as a suitable case to investigate genetic diversity within populations and provenance variation of a vulnerable wind-pollinated conifer. New information would also improve the basis for legal enforcement and protection of this conifer. The long-term benefits of conservation management of *A. religiosa* subsp. *mexicana* in Central America would include protection of unique highland forests which provide local communities with drinking water and protect against landslides (Veblen 1976, Nelson & Chomitz 2004).

The patchy distribution of *A. religiosa* subsp. *mexicana* in southern Mexico and Guatemala has affected its genetic differentiation (Aguirre-Planter *et al.* 2000). According to this study, genetic diversity within populations is relatively low, while differentiation among populations is higher than observed in other conifers from Mexico. Moreover, some of the populations of *A. religiosa* subsp. *mexicana* studied showed high levels of inbreeding. The patterns of genetic structure seem to be due to past glacial refugia and recent forest fragmentation, as also described for *Pinus chiapensis* and *Fagus grandifolia* var. *mexicana* (Newton *et al.* 2002, Williams-Linera *et al.* 2003, Rowden *et al.* 2004) found in the same region.

The data by Aguirre-Planter *et al.* (2000) referred to above were collected from three populations of *A. religiosa* subsp. *mexicana* in Guatemala and seven in Mexico and the results were based on isozyme markers which have relatively low genetic resolution. Thus, it is thought that more sample populations and a method

with higher resolution are needed to develop an improved conservation strategy for this conifer.

The present study used microsatellite data from 18 populations of *A. religiosa* subsp. *mexicana* in the highlands of Guatemala. The objectives were to (1) investigate genetic diversity within and among populations; (2) relate genetic diversity to geographic location, altitude and population size, degree of isolation and fitness traits obtained in associated studies; and (3) estimate the number of 'true' populations in this wind-pollinated species. The results are discussed with respect to management and conservation strategies in this vulnerable conifer.

MATERIALS AND METHODS

Study species

Abies religiosa subsp. *mexicana* (Pinaceae) is a tall conifer (height 35–40 m; diameter at breast height 1.0–1.5 m). This subspecies is endemic to Mexico and Guatemala with possible remnant populations at the border between Honduras and El Salvador (Figure 1). It is the southernmost subspecies within the genus *Abies*, reaching latitude 14° 30' N in the Guatemalan highlands at 1200–4100 m altitude. In Guatemala, montane conifer forests with *A. religiosa* subsp. *mexicana* cover about 26 000 ha (Andersen *et al.* 2006). In these forests, the plant often co-occurs with other tree species, mainly conifers; monospecific stands are rare but have been observed in the departments of Huehuetenango and San Marcos, including some of the study populations (U Strandby, personal observation).

Little is known about the pollination and seed dispersal of *A. religiosa* subsp. *mexicana* but there are some accounts on seed production and germination. Germination of fresh seeds is relatively low, i.e. < 15% (Andersen *et al.* 2008a, b) but this might be compensated for by high seed production. In most forests, old individuals prevail and regeneration is rare and patchy (Standley & Steyermark 1958, Veblen 1976, Kollmann *et al.* 2008), although high densities of young plants have been observed in remote stands with little human influence.

The distribution of *A. religiosa* subsp. *mexicana* has changed markedly over the past 50 years. In the 1940s it was still widespread and locally common in Guatemala and Mexico, while in the late 1950s most remaining stands were heavily exploited, except a few forests on national land where cutting had been prohibited (Standley & Steyermark 1958). The wood has been used for construction purposes, shingles, tools and charcoal. More recently, a Christmas tree market has been established in Guatemala and branches are harvested from natural forests to construct 'artificial' Christmas trees (Strandby & Olsen 2008). Today all *A. religiosa* subsp. *mexicana* forests are protected in Guatemala and cutting for any purpose is prohibited (INAB 1999). Guatemalan fir is included by FAO (1986) and it is listed as 'vulnerable' by IUCN (2009), based on recommendations by the Conifer Specialist Group. However, illegal greenery harvest has increased in the past decade (Strandby & Olsen 2008) and has become a serious threat (together with grazing) to the remaining stands.

Due to the steep topography and considerable deforestation in the highlands of Guatemala most populations of *A. religiosa* subsp. *mexicana* are fragmented. Fragment size is 522 ± 203 ha (mean ± SE; N = 55; range 5–9397 ha; Andersen *et al.* 2006). The largest continuous populations in Guatemala are found in the Municipalities of Totonicapán (about 16 500 ha, close to study population TO (Table 1, Figure 1) and Todos Santos (2700 ha, corresponding to LC and PC, and close to TS), and in the mountain range of Sierra de las Minas (1300 ha, close to SM).

Study populations, leaf samples and laboratory analyses

In the winter of 2004/2005, needles were collected in 18 populations of *A. religiosa* subsp. *mexicana* in Guatemala. The populations were sampled in natural forests in the northern and southern mountain ranges of the country including two peripheral populations in eastern Guatemala (Figure 1). The populations cover a representative altitudinal range (2600–3500 m) with large variation in forest size (5–10 000 ha) and population density (< 50 to > 500 trees ha^{-1}), as well as some differences in geographic isolation (2.4–73.3 km, Table 1). Relative isolation was measured as distance from the fifth closest *A. religiosa* subsp. *mexicana* population, since most study populations (except MA and SM) had up to four stands close by. This simple index produced similar results as other methods tried, e.g. Hanski's connectivity measure. Three populations (SM, MA, TO) were identical to those studied by Aguirre-Planter *et al.* (2000).

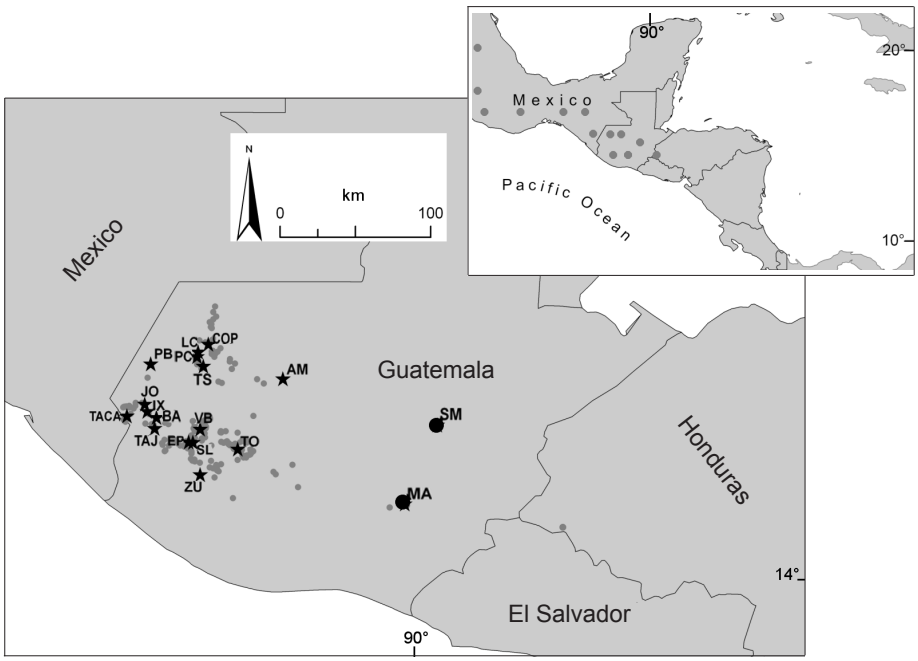

Figure 1 Study populations of *Abies religiosa* subsp. *mexicana* (large black dots) and total distribution of all known populations (small grey dots) in the highlands of Guatemala. Most populations are found within the two main mountain ranges of the country, while the populations MA and SM occur on rather isolated mountains at the eastern distribution margin of the species. The inset map shows the total distribution of *A. religiosa* subsp. *mexicana*. Results of TESS clustering suggest one large metapopulation (stars) across the two mountain ranges, and two geographically and genetically separated populations (MA and SM, dots). For abbreviations of the populations see Table 1.

In all populations, 9–19 trees (> 30 years old) were randomly selected with > 50 m distance among individuals. Fresh needles were collected from a total of 301 trees of which 284 produced useful genetic results. Needles were frozen immediately after collection and stored in plastic tubes with silica gel. DNA extraction, development of microsatellite library and analysis of samples for eight microsatellites were carried out by Ecogenics GmbH (Zürich, Switzerland). For a full description of the laboratory methods and results see Rasmussen *et al.* (2008).

Eight primer pairs gave reproducible and interpretable polymerase chain reaction (PCR) products; the number of alleles ranged from 12 to 26 (Rasmussen *et al.* 2008). Observed and expected heterozygosities over all loci ranged from 0.56 to 0.94 and 0.49 to 1.00 respectively. Four loci showed significant deficit of heterozygotes which indicated inbreeding or presence of null-alleles. Locus *Abgu23* gave an exceptionally high estimated frequency of null-alleles and also produced a rather unclear genetic signal with many stutter bands (Rasmussen *et al.* 2008). Descriptive genetic analyses with and without *Abgu23* revealed that this locus caused a significantly higher proportion of homozygotes and, therefore, also larger estimated inbreeding and different results in all further analyses. We therefore decided to exclude this locus from the study and to report only results based on the remaining seven loci.

Data analyses

Observed and expected heterozygosities were estimated for each locus and all populations as measures of intra-population genetic diversity and allele frequencies. Fisher's exact test was used to assess deviation from Hardy–Weinberg equilibrium as well as linkage disequilibrium (Raymond & Rousset 1995, Lewis & Zaykin 2001). F-statistics were applied as a measure of inbreeding within populations as well as population differentiation and significance was tested by bootstrapping/randomisation test (Goudet 1995, Lewis & Zaykin 2001). Pearson product–moment correlations were calculated between genetic diversity (H_e, N_a, F_{is}) and nine population characteristics (latitude, longitude, altitude, population size and density, degree of isolation, seed mass, germination percentage and

seedling survival); the last three were based on a common garden experiment in the study area including nine of the study populations, i.e. BA, EP, IX, JO, MA, PC, SL, TO, VB (Andersen *et al.* 2008b). No Bonferroni corrections were applied in the present study (see Moran 2003).

At the population level, isolation by distance was analysed by a Mantel test with log-transformed geographic distances and Nei's genetic distance (Peakall & Smouse 2006). Possible explanations for genetic differentiation among populations were investigated by principal component analysis (PCA) (Ihaka & Gentleman 1996) based on a covariance matrix among all individuals (Dyer 2007) and the axes were correlated with the population characteristics 'mountain range', 'altitude', 'degree of isolation', 'forest size' and '*A. religiosa* subsp. *mexicana* density' (classified as 1, < 50; 2, 50–100; 3, 100–500; 4, > 500 trees ha^{-1}; cf. Table 1).

Table 1 Characteristics of the 18 study populations of *Abies religiosa* subsp. *mexicana* in the highlands of Guatemala

Location (department)	Code	Latitude Longitude	Altitude (m asl)	Isolation (km)	Forest size (ha)	Tree density (ha^{-1})
Astillero Municipal in Cunén (Huehuetenango)	AM	15° 23' N 91° 00' W	2800#	39.0	498	100–500
Buenos Aires (San Marcos)	BA	15° 07' N 91° 52' W	3068	7.9	5	> 500
Soloma (Huehuetenango)	COP	15° 38' N 91° 31' W	3096	6.1	15	100–500
El Eden Palestina de los Altos (Quetzaltenango)	EP	14° 57' N 91° 39' W	2865	3.9	62	100–500
Ixchiguán (San Marcos)	IX	15° 10' N 91° 56' W	3381	8.5	73	> 500
San José Ojetenan (San Marcos)	JO	15° 13' N 91° 57' W	3232	7.3	26	> 500
Las Canoa (Huehuetenango)	LC	15° 35' N 91° 35' W	3249	6.1	4	> 500
La Soledad (Jalapa)	MA	14° 31' N 90° 08' W	2600	64.9	6	< 50
Montaña Peña Blanca (Huehuetenango)	PB	15° 30' N 91° 55' W	3320	33.4	50	100–500
Puerta del Cielo (Huehuetenango)	PC	15° 33' N 91° 36' W	3330	3.6	666	> 500
La Laguna Sibilia (Quetzaltenango)	SL	14 57' N 91 37' W	3102	4.1	40	100–500
Sierra de las Minas (El Progreso)	SM	15° 04' N 89° 55' W	2784	73.3	1292	50–100
Volcán Tacaná (San Marcos)	TACA	15° 08' N 92° 05' W	3305	4.7	30	100–500
Volcán Tajumulco (San Marcos)	TAJ	15° 03' N 91° 53' W	3504	3.8	13	100–500
Totonicapán (Totonicapán)	TO	14° 54' N 91° 18' W	3100	3.5	10 000	100–500
Todos Santos (Huehuetenango)	TS	15° 29' N 91° 33' W	2955	7.8	666	> 500
San Vicente Buenabaj (Quetzaltenango)	VB	15° 02' N 91° 34' W	3122	2.4	383	100–500
Zunil, Parque (Quetzaltenango)	ZU	14° 43' N 91° 28' W	3273	11.0	126	< 50

Isolation is calculated as distance to the fifth closest population in Guatemala and adjacent Mexico (populations ordered alphabetically after abbreviation codes); #altitude estimated from Google Earth

Since several populations were relatively small and geographically close to each other, we investigated the number of 'true' (separate) populations to determine the major clusters of genetic diversity. Bayesian clustering method was used to derive the most likely number of genetic clusters. The program STRUCTURE (Pritchard *et al.* 2000) gives a log probability of data for every number of K (L(K)) groups specified, and the K with the largest probability can be taken as the most likely number of clusters. The program was run with default settings, i.e. admixture model and correlated allele frequencies using 10 000 of each burn-in, and Markov Chain Monte Carlo after burn-in (for methodological details see Pritchard *et al.* 2007). Results were compared both in terms of L(K) and ΔK as described by Evanno *et al.* (2005). To improve the robustness of results, the program TESS for spatial Bayesian clustering (Chen *et al.* 2007) was used to compare the estimated clusters including geo-referenced individual population data. This program was run with the admixture model, 50 000 sweeps and burn-in period of 10 000 sweeps. Then the number of estimated clusters (K) from the run with the highest average log-likelihood was selected and this K was repeated 100 times. Then the 15 runs with the highest average log-likelihood were selected and exported for post-processing in the software CLUMPP (Jakobsson & Rosenberg 2007) where the optimal alignment of the K clusters was found by permutation.

RESULTS

Genetic diversity within populations

The seven microsatellite markers used for *A. religiosa* subsp. *mexicana* in this study were highly polymorphic and the average number of alleles per locus across all populations was 28.6, while it ranged from 8.3 to 14.1 within populations (Table 2). Genetic diversity was high in all populations with observed heterozygosity ranging from 0.70 to 0.90. Homozygosity was higher than expected in the six populations and thus deviation from Hardy–Weinberg equilibrium was significant (COP, LC, MA, PC, SM, TO). Genetic diversity (N_a) was higher in the northern populations (Pearson product–moment correlations; r = 0.51, p = 0.032) and in forest stands with high density of *A. religiosa* subsp. *mexicana* trees (r = 0.62, p = 0.006).

The inbreeding coefficient within populations (F_{is}) was significantly larger than zero in five populations (EP, LC, MA, SM, TACA; overall F_{is} = 0.047, 95% CL = 0.021–0.076), and it was positively correlated with geographic isolation (r = 0.51, p = 0.032). After removing the effect of Hardy–Weinberg disequilibrium there was still linkage disequilibrium between loci-pairs in some populations, COP being the most extreme (see Table 2).

Genetic diversity and population characteristics

There was a positive correlation between genetic and geographic distance among all populations (Mantel test; slope = 0.75, p = 0.001). When the two peripheral populations (MA and SM) were removed, the positive correlation was still significant (p = 0.03). Population assignment analysis indicated the presence of some extent of gene flow among all populations; the range of local assignment percentage was 27–90%. The population TAJ had the highest local assignment of 90%, followed by MA (89%), EP (88%) and SM (79%). In the lower end were TACA (27%) and LC (29%) indicating substantial gene flow from other populations (Table 2).

The first two axes of a PCA using an individual-based covariance matrix explained 24% of the overall genetic variance (Figure 2). The populations MA, SM, PB and TAJ were outliers, whereas the other populations were rather similar. The first PCA axis correlated significantly with population longitude (r = -0.58, p = 0.012), geographic isolation (r = -0.52, p = 0.026) and expected heterozygosity (r = 0.57, p = 0.014), while the second PCA axis correlated significantly only with altitude (r = 0.56, p = 0.015). No significant relationship was found with latitude, population size and tree density, seed mass, germination percentage and seedling survival of the various provenances (p > 0.05).

Provenance variation among populations

Clustering analysis based on Nei's genetic distance sorted populations into one outlier (TAJ) and two main clusters, with the peripheral populations AM, MA and SM in the first cluster, and the remaining 13 populations in the second cluster (Figure 3). The latter had the populations PB and ZU as outliers, and then split in three

Table 2 Genetic variation within and among populations of *Abies religiosa* subsp. *mexicana* in Guatemala

Population	N	N_a	H_o	H_e	F_{is}	Disequilibrium	Assignment
AM	14	10.8 (1)	0.832	0.822ns	-0.013ns	5	73
BA	19	12.4	0.865	0.878ns	0.016ns	1	68
COP	16	11.9 (1)	0.848	0.842**	-0.008ns	18	50
EP	17	10.1 (1)	0.807	0.868ns	0.073*	0	88
IX	17	12.1 (1)	0.840	0.885ns	0.052ns	0	65
JO	18	14.1 (0)	0.897	0.903ns	0.007ns	0	47
LC	17	13.6 (4)	0.775	0.865**	0.107**	6	29
MA	18	8.6 (2)	0.698	0.780**	0.107**	3	89
PB	14	10.7 (0)	0.776	0.802ns	0.034ns	0	71
PC	17	13.4 (3)	0.882	0.919***	0.041ns	6	35
SL	19	12.0 (2)	0.857	0.873ns	0.018ns	3	68
SM	19	11.3 (3)	0.722	0.830**	0.133***	1	79
TACA	15	13.9 (4)	0.838	0.907ns	0.079**	0	27
TAJ	10	8.3 (1)	0.786	0.830ns	-0.010ns	0	90
TO	15	10.9 (2)	0.848	0.879*	0.037ns	1	73
TS	11	10.7 (1)	0.861	0.900ns	0.045ns	0	42
VB	16	11.4 (0)	0.854	0.879ns	0.03ns	1	44
ZU	9	8.3 (2)	0.810	0.849ns	0.049ns	0	44

Population order follows the codes explained in Table 1; number of trees sampled (N), average number of alleles over all loci (N_a) with number of unique alleles in brackets, observed (H_o) and expected (H_e) heterozygosities (including significance of deviation from Hardy–Weinberg equilibrium; exact test), fixation index within populations (F_{is}), number of loci-pairs in linkage disequilibrium and percentage of local assignment (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, $p > 0.05$)

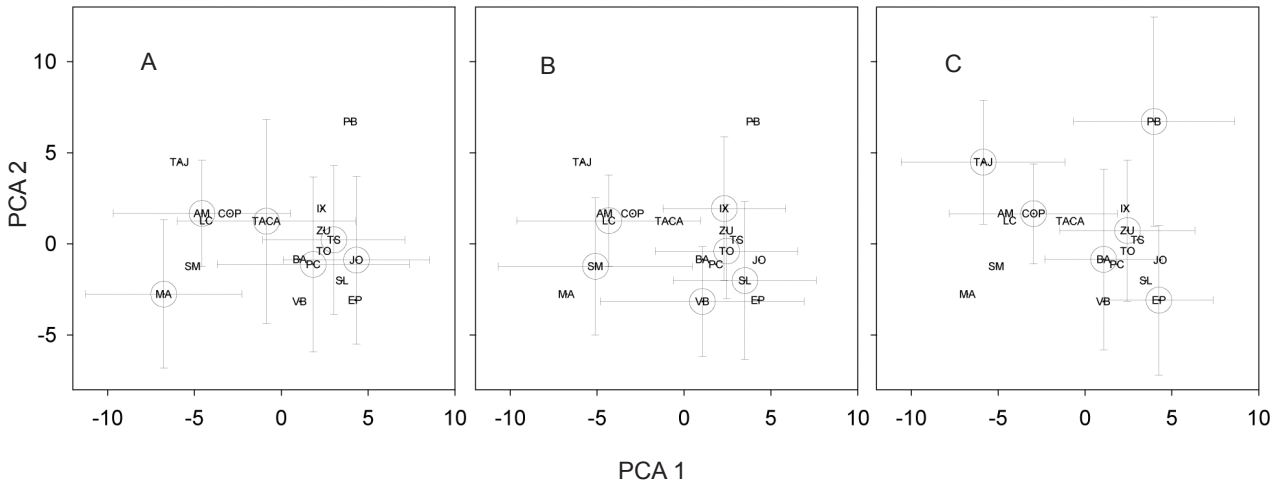

Figure 2 Distribution of the 18 populations of *A. religiosa* subsp. *mexicana* along two PCA axes describing genetic diversity. Population codes indicate population means, while error bars show standard deviation of individual scores for six populations per figure (Figure 2a: AM, JO, MA, PC, TACA, TS; Figure 2b: IX, LC, SM, SL, TO, VB; and Figure 2c: BA, COP, EP, PB, TAJ, ZU; populations were split up to increase readability—abbreviations follow Table 1). PCA 1 correlated negatively with longitude and geographic isolation of the populations, and positively with H_e; PCA 2 correlated positively with altitude (see results section for r and p values).

main clusters which included two population groups from the southern volcanic mountain range and one from the northern Cordillera de los Cuchumatanes. The latter group included one population from the southern mountain range close to the Mexican border (TACA).

The Bayesian clustering procedures used to analyse the number of true populations gave contrasting results. STRUCTURE found no division within the data set; the estimated L(K) was highest at K = 1, and ΔK showed no trend in response to K. Results based on TESS showed two distinct genetic groups and the 15 best runs resulted in one optimal permuted grouping, assigning the populations MA and SM to one group and the remaining populations to a single large group (Figure 1). All populations had an assignment percentage among individual samples of > 70% to one of the two groups. Looking at K = 2 from STRUCTURE (ignoring non-significance) revealed that the grouping here resulted also in one large 'metapopulation' with MA and SM as outlier group and LC and VB with undecided relationship to the two groups.

Evidence for the existence of a single large metapopulation with two geographically distant outlier populations was also found using other analyses of population differentiation. The F_{st} values for all population pairs were relatively low, although the range was large (overall F_{st} = 0.051, 95% CL = 0.041–0.061, range = 0.006–0.128; Table 3). Population means supported the existence of one metapopulation with values lower than the overall F_{st} and a number of more differentiated populations including MA, PB, SM and TAJ.

DISCUSSION

Evaluation of genetic diversity in *A. religiosa* subsp. *mexicana*

Using the above methods, we found relatively high genetic diversity within the study populations both in terms of number of alleles and degree of heterozygosity. This is in contrast to the study by Aguirre-Planter *et al.* (2000) in which isozyme markers were used showing lower

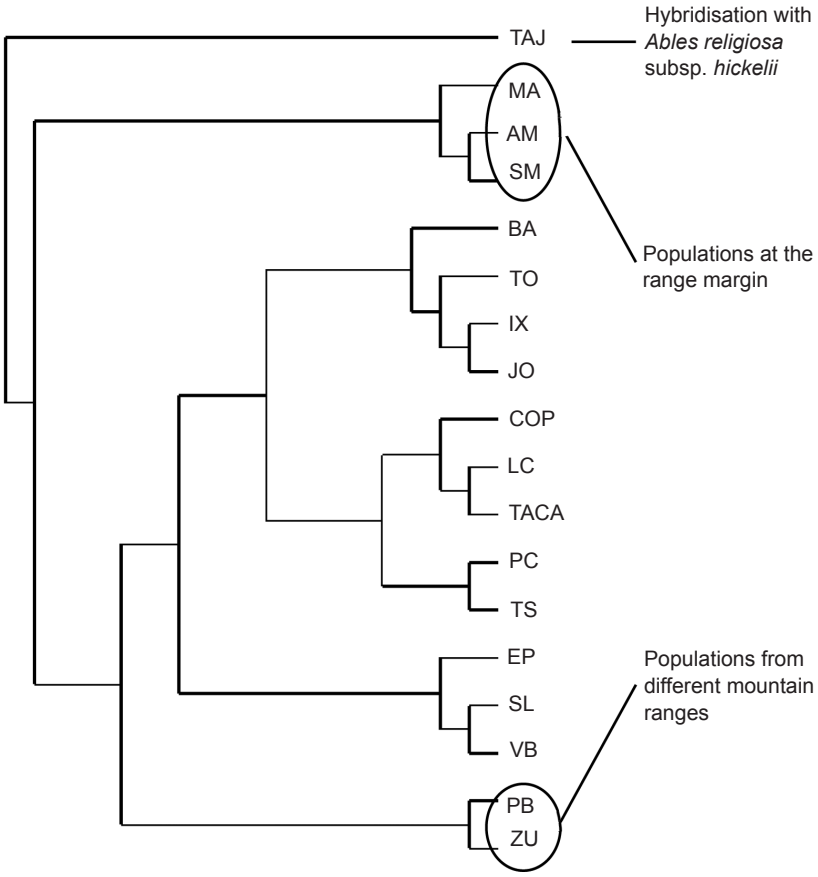

Figure 3 Dendrogram showing similarities among populations of *Abies religiosa* subsp. *mexicana* based on Nei's genetic distance, including some biogeographical interpretation of the similarity pattern, including hybridisation with *A. religiosa* subsp. *hickelii*

Table 3 Genetic differentiation (F_{st}) for all pairs of populations and distance among populations (km, in italics)

	AM	BA	COP	EP	IX	JO	LC	MA	PB	PC	SL	SM	TACA	TAJ	TO	TS	VB	ZU
AM		0.063	0.054	0.078	0.058	0.062	0.019	0.082	0.089	0.061	0.074	0.040	0.039	0.063	0.060	0.057	0.054	0.082
BA	*111*		0.059	0.045	0.039	0.028	0.045	0.077	0.063	0.026	0.031	0.063	0.021	0.073	0.029	0.037	0.035	0.065
COP	*56*	*69*		0.071	0.038	0.058	0.009	0.087	0.082	0.031	0.053	0.054	0.022	0.064	0.042	0.043	0.028	0.058
EP	*105*	*31*	*77*		0.047	0.036	0.056	0.103	0.071	0.036	0.033	0.067	0.043	0.115	0.041	0.040	0.029	0.055
IX	*115*	*8*	*69*	*39*		0.016	0.027	0.101	0.056	0.021	0.040	0.065	0.019	0.056	0.028	0.029	0.036	0.042
JO	*114*	*14*	*67*	*44*	*6*		0.044	0.101	0.060	0.019	0.026	0.066	0.022	0.076	0.021	0.022	0.025	0.059
LC	*64*	*60*	*10*	*70*	*60*	*195*		0.066	0.078	0.018	0.051	0.034	0.006	0.041	0.039	0.025	0.030	0.056
MA	*156*	*198*	*193*	*169*	*206*	*210*	*195*		0.126	0.070	0.088	0.049	0.057	0.127	0.095	0.089	0.079	0.075
PB	*100*	*42*	*46*	*67*	*37*	*31*	*37*	*219*		0.070	0.062	0.094	0.049	0.109	0.065	0.056	0.073	0.070
PC	*65*	*56*	*13*	*66*	*57*	*54*	*4*	*193*	*35*		0.032	0.044	0.008	0.069	0.029	0.009	0.020	0.048
SL	*103*	*33*	*77*	*3*	*42*	*47*	*70*	*166*	*69*	*67*		0.062	0.037	0.109	0.029	0.031	0.023	0.048
SM	*133*	*211*	*183*	*187*	*218*	*220*	*188*	*66*	*220*	*188*	*184*		0.033	0.089	0.062	0.064	0.035	0.057
TACA	*130*	*22*	*83*	*50*	*16*	*16*	*73*	*220*	*44*	*70*	*54*	*233*		0.049	0.026	0.020	0.018	0.036
TAJ	*117*	*9*	*77*	*28*	*14*	*20*	*68*	*197*	*50*	*64*	*31*	*212*	*23*		0.084	0.082	0.082	0.128
TO	*90*	*66*	*84*	*38*	*74*	*78*	*81*	*132*	*93*	*78*	*35*	*150*	*87*	*65*		0.034	0.027	0.057
TS	*63*	*53*	*18*	*60*	*55*	*53*	*12*	*185*	*39*	*9*	*60*	*181*	*69*	*60*	*69*		0.030	0.059
VB	*92*	*34*	*66*	*13*	*42*	*46*	*60*	*164*	*63*	*56*	*12*	*178*	*56*	*34*	*32*	*49*		0.045
ZU	*116*	*63*	*102*	*33*	*72*	*77*	*97*	*144*	*99*	*93*	*31*	*171*	*81*	*59*	*27*	*85*	*38*	
Mean	0.061	0.047	0.050	0.057	0.042	0.044	0.038	0.087	0.075	0.036	0.049	0.058	0.030	0.083	0.045	0.043	0.039	0.061

genetic diversity in *A. religiosa* subsp. *mexicana* compared with other *Abies* species; *A. religiosa* subsp. *mexicana* had a particularly low number of alleles, low heterozygosity as well as the highest fixation index. However, the results of the present study were not directly comparable with those of Aguirre-Planter *et al.* (2000) since it used molecular markers with higher resolution. Moreover, the present study showed that two of the populations investigated by Aguirre-Planter *et al.* (2000) in Guatemala (SM, MA) were rather isolated and genetically poor. This suggests that the previous study may have underestimated the overall genetic diversity of *A. religiosa* subsp. *mexicana* in Guatemala due to sparse and non-representative sampling.

Positive correlation between genetic diversity and fitness is considered a general rule and has been found in numerous studies (Leimu *et al.* 2006, and references within), although there are also examples of negative correlations between genetic diversity and fitness. However, in the present study genetic diversity was not correlated with three fitness components studied in a common garden experiment, namely, seed mass, seed germination and seedling survival (Andersen *et al.* 2008b). This indicated that phenotypic differences in *A. religiosa* subsp. *mexicana* were not due to genetic erosion at least in some of the study populations but that they more likely reflect specific adaptations to local site conditions. However, the present study investigated adult individuals and in small and isolated populations, genetic diversity of seedlings may be different from adults (Premoli *et al.* 2007).

Genetic diversity in *A. religiosa* subsp. *mexicana* increased with tree density and it decreased with isolation of populations. However, although the smallest populations (MA, SM, ZU) were genetically most distinct, there was no support for another general assumption in population genetics, namely, that there is generally a positive correlation between population size and genetic diversity (Leimu *et al.* 2006). In the present study, the largest populations (TO, LC, PC, SM) showed deviation from Hardy–Weinberg equilibrium and two of these populations (LC, SM) had inbreeding depression. This might partly be due to insufficient estimates of population size (Table 2) but could also be explained by recent fragmentation of the study populations as also noted by Premoli *et al.* (2007). Thus, in the highlands of Guatemala not enough time has elapsed to produce significant effects of fragmentation on genetic diversity of *A. religiosa* subsp. *mexicana*.

Provenance differentiation and regional biogeography

We had expected some genetic differentiations between *A. religiosa* subsp. *mexicana* populations from the two main mountain ranges in Guatemala, as described for other tree species under similar geographic conditions in the highlands of Mexico and Chile (Premoli *et al.* 2007). However, the majority of the 18 study populations seemed to constitute one metapopulation with substantial gene flow, resulting in non-local ancestry of up to 73% of the individuals in one population. The Bayesian clustering methods disagreed slightly with this result but showed clearly that the two peripheral populations, MA and SM, were outliers. This agrees with the genetic boundary within Guatemala described by Jaramillo-Correa *et al.* (2008). The results also support the assumption that fragmentation of *A. religiosa* subsp. *mexicana* populations in Central America has been a rather recent process, and that glacial refugia have not played the same role in this conifer as it has done in the tree species described by Premoli *et al.* (2007). Similar observations as in our study were reported for recently fragmented *Abies* species in the western Mediterranean Basin (Terrab *et al.* 2007). Another explanation could be long-distance pollen dispersal which is known to facilitate gene flow in conifers over large distances, possibly over 200 km (Liepelt *et al.* 2002). Strong north-eastern trade winds in Guatemala could explain the close relatedness among some populations from the northern and southern mountain range studied (Figure 3).

Results of this study also indicated that there was genetic differentiation among populations in agreement with isolation-by-distance models, as described for other neotropical tree species by Premoli *et al.* (2007). The correlation between geographic and genetic distance, which was supported by results of the PCA, was independent of the two outlier populations, MA and SM. These populations were relatively isolated and small and they showed low genetic diversity, high inbreeding and marked genetic differentiation. Common garden experiments with seeds from MA gave low germination (Andersen *et al.* 2008b) and no natural regeneration was observed in this population (Kollmann *et al.* 2008). Thus,

viability of these populations might be low. This supports the central-marginal model of plant distribution (Brussard 1984, Siikamäki & Lammi 1998, Eckert *et al.* 2008) and it underlines the need to include peripheral populations when considering conservation of genetic diversity of endangered species (Lesica & Allendorf 1995).

Overall, there was a clear relation between geographic isolation and increased inbreeding within populations. However, we know little about the history of most populations and therefore cannot explain the high fixation index in some populations, for example LC, which is not geographically isolated. In addition, positive correlation between genetic diversity and altitude was observed, as reflected by the correlation with the second PCA axis.

Management implications

The patterns of genetic diversity detected in this study can be used to guide and support conservation planning of *A. religiosa* subsp. *mexicana* in Guatemala, although some genetic diversity remains unexplained due to the largely unknown biogeographic and evolutionary history of this subspecies. The results suggest that several populations should be included in conservation action, if the full variation within *A. religiosa* subsp. *mexicana* is to be conserved. Most importantly, the two isolated populations (MA and SM) contained unique alleles and showed inbreeding depression. To conserve these genotypes and facilitate natural regeneration in the respective populations, these forests should be protected from cutting and grazing. In addition, *ex situ* conservation is advisable to support *in situ* measures, since the MA and SM populations are confined to isolated mountain tops with high risk of further depletion or extinction, for example as a consequence of regionally increasing temperatures.

Interestingly, most of the central populations of *A. religiosa* subsp. *mexicana* in Guatemala still show high genetic variation and substantial gene flow, despite widespread habitat fragmentation. Possibly, wind dispersal of pollen secures genetic exchange among relatively isolated populations. Although the consequences of recent fragmentation and illegal cutting are unknown, and although fragmentation probably has reduced the reproductive success of *A. religiosa* subsp. *mexicana*, it seems that most populations of this vulnerable conifer species presently suffer no major genetic threat. The focus of conservation should therefore be on preventing further direct damage to the existing populations and to collect genetic material from central as well as from peripheral populations for complementary *ex situ* conservation measures.

Conservation and genetic management of Guatemalan fir should be incorporated in wider planning frameworks, such as national forest programmes and rural development plans (Palmberg-Lerche 2008), especially in species with commercial value.

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REFERENCES

Aguirre-Planter E, Furnier GR & Eguiarte LE. 2000. Low levels of genetic variation within and high levels of genetic differentiation among populations of species of *Abies* from Southern Mexico and Guatemala. *American Journal of Botany* 87: 362–371.

Allendorf FW & Luikart G. 2007. *Conservation and the Genetics of Populations*. Blackwell Publishing, Malden.

Andersen US, Córdova JP, Sørensen M & Kollmann J. 2006. Conservation and utilisation of *Abies guatemalensis* Rehder (Pinaceae)—an endangered endemic conifer in Central America. *Biodiversity and Conservation* 15: 3131–3151.

Andersen US, Córdova JP, Nielsen UB, Olsen CS, Nielsen C, Sørensen M & Kollmann J. 2008a. Conservation through utilization: a case study of the vulnerable *Abies guatemalensis* in Guatemala. *Oryx* 42: 206–213.

Andersen US, Córdova JP, Nielsen UB & Kollmann J. 2008b. Provenance variation in germination and seedling growth of *Abies guatemalensis* Rehder. *Forest Ecology and Management* 255: 1831–1840.

Brussard PF. 1984. Geographic patterns and environmental gradients: the central-marginal model in *Drosophila* revisited. *Annual Review of Ecology and Systematics* 15: 25–64.

Chen C, Durand E, Forbes F & Francois O. 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes* 7: 747–756.

Cornman RS & Arnold ML. 2007. Phylogeography of *Iris missouriensis* (Iridaceae) based on nuclear and chloroplast markers. *Molecular Ecology* 16: 4585–4598.

Donahue JK, Dvorak W, Guitierrez E & Kane M. 1985. Abies guatemalensis: *A Two Year Status Report.* CAMCORE Bulletin on Tropical Forestry No. 3. North Carolina State University, Raleigh.

Dyer RJ. 2007. *Genetic Studio.* http://dyerlab.bio.vcu.edu/trac/GeneticStudio. (Accessed October 2008)

Eckert CG, Samis KE & Lougheed SC. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* 17: 1170–1188.

Eckstein RL, O'Neill RA, Danihelka J, Otte A & Koehler W. 2006. Genetic structure among and within peripheral and central populations of three endangered floodplain violets. *Molecular Ecology* 15: 2367–2379.

Ellstrand NC & Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.

Echeverría C, Cayuela L, Manson RH, Coomes DA, Lara A, Rey-Benayas JM & Newton AC. 2007. Spatial and temporal patterns of forest loss and fragmentation in Mexico and Chile. Pp. 14–42 in Newton AC (Ed.) *Biodiversity Loss and Conservation in Fragmented Forest Landscapes: The Forests of Montane Mexico and Temperate South America.* CAB International, Wallingford.

Evanno G, Regnaut S & Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620.

FAO (Food and Agriculture Organisation) 1986. *Databook on Endangered Tree and Shrub Species and Provenances.* FAO Forestry Paper 77. FAO, Rome.

Fischer M & Matthies D. 1998. RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *American Journal Botany* 85: 811–819.

Fischer M, Hock M & Paschke M. 2003. Low genetic variation reduces cross-compatibility and offspring fitness in populations of a narrow endemic plant with a self-incompatibility system. *Conservation Genetics* 4: 325–336.

Galeuchet DJ, Perret C & Fischer M. 2005. Microsatellite variation and structure of 28 populations of the common wetland plant, *Lychnis flos-cuculi* L., in a fragmented landscape. *Molecular Ecology* 14: 991–1000.

Gaston KJ. 2003. *The Structure and Dynamics of Geographic Ranges.* Oxford University Press, Oxford.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86: 485–486.

Hamrick JL, Godt MJW & Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in woody plant species. Pp. 95–124 in Adams WT *et al.* (Eds.) *Population Genetics in Forest Trees.* Kluwer Academic Publishing, Boston.

Hensen I & Oberprieler C. 2005. Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in central Germany. *Conservation Genetics* 6: 63–73.

Honnay O & Jacquemyn H. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21: 823–831.

Ihaka R & Gentleman R. 1996. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5: 299–314.

INAB (Instituto Nacional de Bosques). 1999. *Diagnóstico de las Poblaciones Naturales de Pinabete (*Abies guatemalensis *R.) en Guatemala y Estrategia para su Conservación.* INAB, Guatemala.

IUCN (International Union for Conservation of Nature). 2009. *IUCN Red List of Threatened Species.* http://www.iucnredlist.org/. (Accessed August 2009)

Jacquemyn H, Vandepitte K, Brys R, Honnay O & Roldan-Ruiz I. 2007. Fitness variation and genetic diversity in small, remnant populations of the food deceptive orchid *Orchis purpurea. Biological Conservation* 139: 203–210.

Jakobsson M & Rosenberg N. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801–1806.

Jaramillo-Correa JP, Aguirre-Planter E, Khasa DP, Eguiarte LE, Pinero D, Furnier GR & Bousquet J. 2008. Ancestry and divergence of subtropical montane forest isolates: molecular biogeography of the genus *Abies* (Pinaceae) in southern Mexico and Guatemala. *Molecular Ecology* 17: 2476–2490.

Kollmann J, Córdova JPP & Andersen RM. 2008. Factors limiting regeneration of an endangered conifer in the highlands of Guatemala. *Journal of Nature Conservation* 16: 146–156.

Leimu R, Mutikainen P, Koricheva J & Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94: 942–952.

Lesica P & Allendorf FW. 1995. When are peripheral populations valuable for conservation? *Conservation Biology* 9: 753–760.

Lewis PO & Zaykin D. 2001. *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data. Version 1.0 (d16c).* http://www.eeb.uconn.edu/people/plewis/software.php. (Accessed October 2008)

Liepelt S, Bialozyt R & Ziegenhagen B. 2002. Wind-dispersed pollen mediates postglacial gene flow among refugia. *Proceedings of the National Academy of Sciences of the United States of America* 99: 14590–14594.

Moran DM. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100: 403–405.

Nelson A & Chomitz KM. 2004. The forest-hydrology-poverty nexus in Central America: an heuristic analysis. *World Bank Policy Research Working Paper* 3430: 1–39.

Newton AC, Allnut TR, Gillies ACM, Lowe AJ & Ennos RA. 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends in Ecology and Evolution* 14: 140–145.

Newton AC, Allnut TR, Dvorak W, del Castillo R & Ennos RA. 2002. Patterns of genetic variation in *Pinus chiapensis,* a threatened Mexican pine, detected by RAPD and mitochondrial DNA RFLP markers. *Heredity* 89: 191–198.

Oostermeijer JGB, Luijten SH, Ellis-Adam AC & den Nijs JCM. 2002. Future prospects for the rare, late-flowering *Gentianella germanica* and *Gentianopsis ciliata* in Dutch nutrient-poor calcareous grasslands. *Biological Conservation* 104: 339–350.

Palmberg-Lerche C. 2008. Thoughts on the conservation of forest biological diversity and forest tree and shrub genetic resources. *Journal of Tropical Forest Science* 20: 300–312.

Peakall R & Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Premoli AC, del Castillo RF, Newton AC, Bekessy S, Caldiz M, Martínez-Araneda C, Mathiasen P, Núñez-Ávila MC, Quiroga P, Souto C & Trujillo-Argueta S. 2007. Patterns of genetic variation in tree species and their implications for conservation. Pp. 120–157 in Newton AC (Ed.) *Biodiversity Loss and Conservation in Fragmented Forest Landscapes: The Forests of Montane Mexico and Temperate South America*. CAB International, Wallingford.

Pritchard JK, Stephens M & Donelly P. 2000. Inference of population structure from multilocus genotype data. *Genetics* 155: 945–959.

Pritchard JK, Wen X & Falush D. 2007. *Documentation for Structure Software: Version 2.2*. University of Oxford, Oxford.

Rasmussen KK & Kollmann J. 2008. Low genetic diversity in small peripheral populations of a rare European tree (*Sorbus torminalis*) dominated by clonal reproduction. *Conservation Genetics* 9: 1533–1539.

Rasmussen KK, Andersen US, Frauenfelder N & Kollmann J. 2008. Microsatellite markers for the endangered fir *Abies guatemalensis* (Pinaceae). *Molecular Ecology Resources* 8: 1307–1309.

Raymond M & Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

Rey-Benayas JM, Cayuela L, González-Espinosa M, Echeverría C, Manson RH, Williams-Linera G, del Castillo RF, Ramírez-Marcial N, Muñiz-Castro MA, Blanco-Macías A, Lara A & Newton AC. 2007. Plant diversity in highly fragmented forest landscapes in Mexico and Chile: implications for conservations. Pp. 43–68 in Newton AC (Ed.) *Biodiversity Loss and Conservation in Fragmented Forest Landscapes: The Forests of Montane Mexico and Temperate South America*. CAB International, Wallingford.

Rowden A, Robertson A, Allnutt T, Heredia S, Williams-Linera G & Newton AC 2004. Conservation genetics of Mexican beech, *Fagus grandifolia* var. *mexicana*. *Conservation Genetics* 5: 475–484.

Siikamäki P & Lammi A. 1998. Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. *Evolution* 52: 1285–1292.

Standley PC & Steyermark JA. 1958. *Abies guatemalensis*. Flora of Guatemala. *Fieldiana, Botany* 24: 37–40.

Strandby U & Olsen CS. 2008. The importance of understanding trade when designing effective conservation policy—the case of the vulnerable *Abies guatemalensis* Rehder. *Biological Conservation* 141: 2959–2968.

Strandby U, Christensen KI & Sørensen M. 2009. A morphometric study of the *Abies religiosa–hickelii–guatemalensis* complex (Pinaceae) in Guatemala and Mexico. *Plant Systematics and Evolution* 280: 59–76.

Terrab A, Talavera S, Arista M, Paun O, Stuessy TF & Tremetsberger K. 2007. Genetic diversity at chloroplast microsatellites (cpSSRs) and geographic structure in endangered West Mediterranean firs (*Abies* spp., Pinaceae). *Taxon* 56: 409–416.

Travis JMJ & Dytham C. 2004. A method for simulating patterns of habitat availability at static and dynamic range margins. *Oikos* 104: 410–416.

Veblen TT. 1976. The urgent need for forest conservation in highland Guatemala. *Biological Conservation* 9: 141–154.

Willi Y, Van Buskirk J & Hoffmann AA. 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* 37: 433–458.

Williams-Linera G, Rowden A & Newton AC. 2003. Distribution and stand characteristics of relict populations of Mexican beech (*Fagus grandifolia* var. *mexicana*). *Biological Conservation* 109: 27–36.