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Historic and recent fragmentation coupled with altitude affect the genetic population structure of one of the world's highest tropical tree line species

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ABSTRACT

Aim To assess the effects of altitude and historic and recent forest fragmentation on the genetic diversity and structure of the wind-pollinated tropical tree line species *Polylepis incana*.

Location One of the highest mountain forest regions of the world, located in the Eastern Cordillera of the Ecuadorian Andes.

Methods We compared genetic diversity and structure of adult trees with those of seedlings (n = 118 in both cases) in nine forest stands spanning an altitudinal gradient from 3500 to 4100 m a.s.l. using amplified fragment length polymorphisms (AFLPs). Genetic diversity was calculated as percentage of polymorphic bands (P) and Nei's expected heterozygosity (He); genetic differentiation was assessed using analysis of molecular variance, Φ_{ST} statistics and Bayesian cluster analysis.

Results Estimates of genetic diversity at the population level were significantly lower in seedlings than in adults. Genetic diversity (He-value) was, in both cases, negatively correlated to altitude and positively correlated to population size in the seedlings. Genetic differentiation of the seedlings was approximately as high ($\phi_{ST} = 0.298$) as that of the adults ($\phi_{ST} = 0.307$), and geographical differentiation was clearly reflected in both AFLP profiles, with mountain ridges acting as barriers to gene flow.

Main conclusions Our study provides evidence of a historic upslope migration of *P. incana* in central Ecuador. In addition, it highlights the detrimental effects of unexpectedly strong genetic isolation, both recent and historical, particularly for our wind-pollinated species where the distance between forest stands was less than 25 km. We therefore additionally propose that in habitats with pronounced high-mountain landscape structures, gene flow may be hampered to such an extent that species have a more pronounced sensitivity to habitat fragmentation, even among populations of wind-pollinated trees.

Keywords

AFLPs, altitudinal gradient, Central Andes, Ecuador, genetic structure, high-mountain forests, life stages, *Polylepis incana*.

INTRODUCTION

Studies have shown that species respond to climate warming by migrating to higher elevations or latitudes (e.g. Parmesan & Yohe, 2003). As such movements are usually associated with founder effects or bottlenecks, information on the current distribution of the genetic diversity of dominant tree species has facilitated our general understanding of phylogeographical patterns, including post-glacial migration events (Hewitt, 2000). However, for tropical regions such as South America, knowledge

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of genetic structure and its effects on tree migration is still patchy, in particular for high-mountain species (Quiroga & Premoli, 2007; Pautasso, 2009). Results of phylogeographical studies of South American mountain forests suggest that dominant tree species retreated to lower elevations during cooler climates and expanded to higher altitudes during warmer periods in response to the Quaternary glaciations and interglacial periods, respectively (Quiroga & Premoli, 2007, 2010). As a consequence, the genetic diversity of the highest forest stands in these studies was reduced. This implies that upslope movements in tree species during the course of climate change may be relatively slow, and that they are associated with declining genetic diversity. As yet, genetic patterns have rarely been studied in tree line stands, which are expected to be most sensitive to the effects of climate change (Jobbágy & Jackson, 2000).

In addition to historical events, recent forest fragmentation is generally assumed to modify genetic connectivity, reduce gene flow between populations (Young *et al.*, 1996; Frankham *et al.*, 2002) and increase the susceptibility of populations to declining genetic diversity associated with small population sizes (Lowe *et al.*, 2005). The degree to which these processes take place partly depends on the biological attributes of the taxa involved: population size and density, generation times and longevity, as well as modes of reproduction (Frankham *et al.*, 2002; Aguilar *et al.*, 2006). In addition, landscape structure is known to influence genetic structure (Sork & Smouse, 2006).

Among trees, which are long-lived organisms, wind-pollinated species of temperate forests in particular have been shown to be relatively resistant to habitat fragmentation due to their high effective gene flow (Hamrick *et al.*, 1992; Kramer *et al.*, 2008). However, for many of the studies, fragmentation is relatively recent, making it difficult to detect effects in such species due to the long lifecycle response times involved. However, negative genetic effects of century-long habitat fragmentation on the population structure of the wind-pollinated *Fagus sylvatica* (Jump & Peñuelas, 2006) and *Taxus baccata* (Dubreuil *et al.*, 2010) in north-eastern Spain have been reported. In both species, habitat fragmentation resulted in genetic bottlenecks, increased levels of inbreeding, high population differentiation and reduced within-population genetic diversity.

In the central Andes, the natural forest vegetation at elevations between 3000 m and 4500–5200 m primarily comprises mono-dominant forests of the genus *Polylepis* (Rosaceae). Today, these trees mostly form isolated forest patches far above the current closed tree line. This particular distribution pattern is expected to have resulted from a combination of climatic changes (Di Pasquale *et al.*, 2008; Gosling *et al.*, 2009) and thousands of years of logging, domestic livestock grazing and use of fire. All of these have been shown to have effects on survival, growth and regeneration in *Polylepis* species (Renison *et al.*, 2006; Cierjacks *et al.*, 2008b). *Polylepis incana* is currently listed as a vulnerable species which is severely affected by burning and cutting for firewood and charcoal production (World Conservation Monitoring Centre, 1998). The results of a genetic study on *Polylepis australis*, the southernmost subtropical species of this genus (I. Hensen *et al.*, pers. comm.) revealed that past fragmentation was counteracted by effective long-distance wind pollination (Seltmann *et al.*, 2007). However, data from two populations (see Julio *et al.*, 2008) also suggest diminished gene flow resulting from more recent fragmentation events.

In the core distribution area of *Polylepis* in the tropical Andes where *Polylepis* species form potentially the most extensive tropical montane forest belt in the world, the high fragmentation of the extant forests may reflect a comparatively old pattern. A recent study suggests that Ecuadorian *Polylepis* forests failed to recolonize high-altitude sites after the Last Glacial Maximum due to recurrent, partly anthropogenic fires (Di Pasquale *et al.*, 2008). An alternative view is based on the current rapid clearance of *Polylepis* forests by humans; implying that forests were more continuous during earlier phases of the Pleistocene (Purcell & Brelsford, 2004). This view is supported by a recent study on allozyme variation of *Polylepis pauta* from 12 forest populations in three adjacent watersheds in north-eastern Ecuador (Aragundi *et al.*, 2011).

For the present study, we assessed patterns of amplified fragment length polymorphism (AFLP) variation in nine populations of *Polylepis incana* in central Ecuador to address possible divergences in genetic diversity (1) between differing altitudes and (2) between different generations. Our baseline hypothesis is that genetic diversity decreases towards higher elevations. We additionally expected that forest fragmentation diminishes gene flow among *P. incana* populations, and that genetic differentiation is higher among populations of seedlings showing the effects of recent (< 100 years) fragmentation than it is for adults forming stands > 100 years old.

MATERIALS AND METHODS

Study species and study area

The genus *Polylepis* (Rosaceae) comprises between 15 and 30 tree and shrub species (Schmidt-Lebuhn *et al.*, 2010) that are endemic to the Andean mountain chain from Argentina and Chile to Venezuela. Their flowers are apetalous, wind-pollinated and proterogynous, and their fruits are one-seeded gravity-dispersed nutlets with a low dispersal capacity (Cierjacks *et al.*, 2007). In addition to sexual regeneration, several species (including *P. incana*; Cierjacks *et al.*, 2007) have the capacity to produce asexual ramets, which sprout from horizontal branches producing rootlets when they come into contact with soil. Our study species *P. incana* is distributed from southernmost Colombia to southern Peru.

The study area is Páramo de Papallacta, located about 30 km east of Quito in the Eastern Cordillera of the Ecuadorian Andes at elevations between 3500 m and 5728 m (Fig. 1). In the study region, *P. incana* grows on relatively dry, west-facing slopes between 3500 m and 4100 m. The fragmentation of these forests in the central Andes presumably began centuries or even millennia ago with the use of fire by early hunters (Kessler, 1995; Chepstow-Lusty *et al.*, 1998), although no detailed data are available for the study region. In central Ecuador, village-based

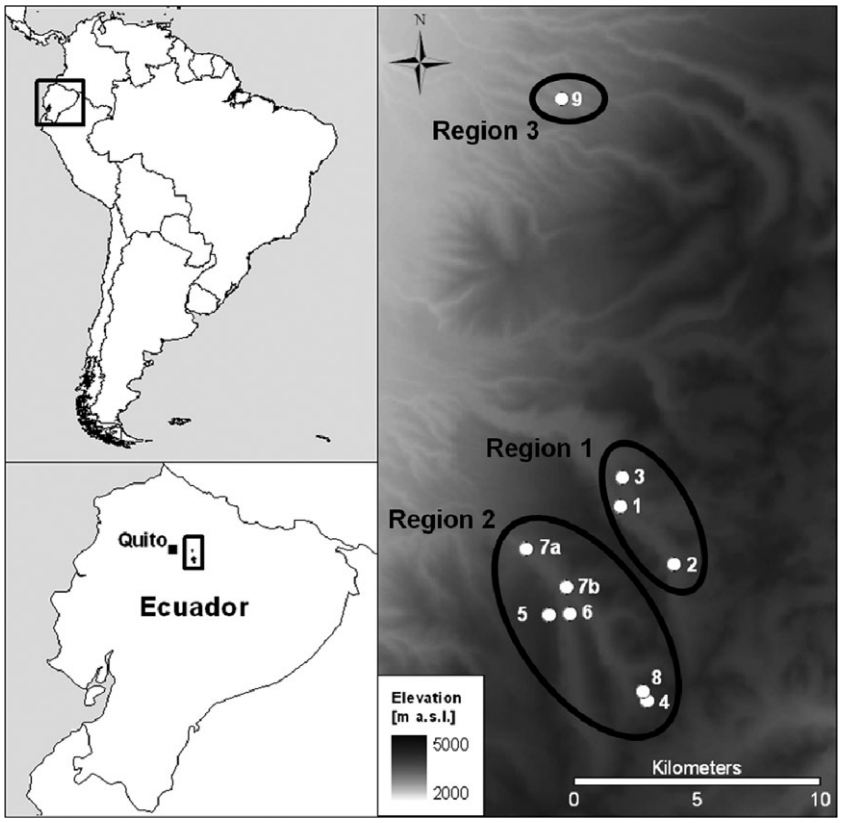

Figure 1 Location of the sampled populations of *Polylepis incana* in Ecuador (ellipses: the three population groups). Sampling is comprehensive, as gaps are not colonized by *P. incana*. Population numbers refer to Table 1.

agro-pastoral economies have existed since 3000–4000 yr BP. The introduction of European land-use practices such as sheep and cattle grazing has heavily accelerated forest destruction in the last few centuries (Cierjacks *et al.*, 2008a).

Sampling

Our study covered the most extensive *P. incana* forest areas in Ecuador. We sampled a total of nine *P. incana* forest remnants, with eight stands being located in the Páramo de Papallacta (3500–4080 m a.s.l.) and one stand in the Mojanda region (3710 m a.s.l.; Table 1, Fig. 1). In Papallacta, populations were located in two different valleys separated by a mountain crest of about 4200 m; one located in the plateau-like Páramo de Papallacta s. str. (Papallacta valley), the other in El Tablon/Páramo de Guamaní (Guamaní valley), which is dominated by glacially formed valleys (Fig. 1). The largest forest (7) was sampled at two locations (7a and 7b), which were 2.3 km apart with 220 m difference in elevation (Table 1). The minimum distance between two forest remnants in the Páramo de Papallacta was 300 m, with the maximum distance being 9.1 km (mean 4.7 km). Distances between these populations and that of Mojanda ranged between 15 and 25 km. Forest fragment sizes varied between 0.8 and 71 ha. Population densities were estimated using the data of Cierjacks *et al.* (2008a) and varied between 250 and 2033 adult *P. incana* trees ha^{-1}. We collected leaf samples from 10 to 13 adults and 10 to 13 seedlings (smaller than 5 cm) per forest fragment, totalling 236 individuals (118 adults and 118 seedlings and mean numbers of 11.8 for adults and seedlings per fragment). Sampled adult trees were separated by at least 20 m to minimize the chance of sampling closely related or genetically identical individuals. Given the size of the adults and estimated growth rates for other species of the genus (Suarez *et al.*, 2008), we assume that the majority of individuals were > 100 years old. Leaves were stored in bags with silica gel prior to analysis. In the small and isolated Mojanda stand we found only four seedlings overall. Therefore, we collected seeds from at least 10 different trees and extracted the DNA from a total of eight seedlings grown in the lab to obtain 12 individuals for the stand.

DNA extraction and AFLP analysis

The extraction method was a standard protocol adapted from Doyle & Doyle (1987) using 20 mg silica-gel-dried leaf material and a modified extraction buffer [2% alkyltrimethylammonium bromide (ATMAB), 0.1 M TRIS–HCl, 0.02 M disodium-EDTA (pH 8.0), 1.4 M NaCl, 1% polyvinylpyrrolidone]. Extracted genomic DNA was double-digested with the restriction enzymes *Mse*I and *Eco*RI, and the ends of the resulting fragments were ligated to double-stranded adapter oligonucleotides (5′-GACGATGAGTCCTGAG-3′/5′-TACTCAGGACTCAT-3′ and 5′-CTCGTAGACTGCGTACC-3′/5′-AATTGGTACGCAGTCTAC-3′) serving as primer binding sites in the following steps. Restriction and ligation were performed for 3 h at 37 °C, followed by 10 min at 65 °C in an 11-µl volume

Table 1 Geographical and genetic data for the sites used for leaf collection of adults (A) and seedlings (S) of *Polylepis incana* (sub)populations in Ecuador.

Population	Region code	Pop. area (ha)	Pop. size	*n* (A)	*n* (S)	Geographical			P%(A)	P%(S)	He(A)	He(S)
						Lt	Ln	Al				
1	1	34	44,200	11	11	00.18	78.14	3650	43.3	37.0	0.150	0.117
2	1	7	9800	12	12	00.19	78.13	3720	44.6	45.8	0.144	0.122
3	1	1	1200	12	12	00.17	78.14	3630	52.1	41.0	0.173	0.127
4	2	8	9600	12	12	00.22	78.14	3930	39.6	39.3	0.131	0.108
5	2	17	30,000	12	13	00.20	78.16	4080	39.2	42.4	0.126	0.117
6	2	3	7000	12	12	00.20	78.15	3750	47.5	35.6	0.150	0.108
7a	2	71	81,700	12	12	00.19	78.16	3500	49.6	50.6	0.160	0.158
7b	2			12	10	00.19	78.16	3720	47.5	35.9	0.153	0.112
8	2	5	4340	12	12	00.22	78.14	3950	41.7	31.9	0.134	0.095
9	3	0.8	200	11	12	00.09	78.16	3710	43.3	26.8	0.133	0.080

Values in the column 'Region code' indicate the regional location of the populations (1, Páramo de Papallacta; 2, Páramo de Guamaní; 3, Mojanda). Symbols of variables: *n*, sample size; Pop. area (ha), area covered with *P. incana* forests; Pop. size, population size; Lt, latitude (decimals); Ln, longitude (decimals); Al, altitude (m a.s.l.); P%, percentage of polymorphism; He, mean gene diversity.

containing 1 µl of *Mse*I (50 µM), 1 µl of *Eco*RI (5 µM), 0.17 µl of T4 DNA ligase (20,000 U), 1.1 µl of T4 DNA ligase 10× reaction buffer (all from New England Biolabs, Frankfurt am Main, Germany), 1.1 µl NaCl (0.5 mM), 0.55 µl bovine serum albumin (BSA), 1 µl of *Eco*RI-adapter (5 pmol), 1 µl *Mse*I adapter (50 pmol) and 5.0 µl DNA extract (20 ng μl^{-1}). The ligation product was diluted with 39 µl of sterile demineralized water and then pre-amplified with the primer combination *Eco*RI + A/*Mse*I + C (E01, 5′-GACTGCGTACCAATTC+A-3′/M02, 5′-GATGAGTCCTGAGTAA+C-3; primer nomenclature following Keygene, 2004). Pre-amplification was performed in a 20-µl volume containing 0.1 µl BioTaq DNA polymerase (5 U μl^{-1}), 2.0 µl PCR 10× reaction buffer, 0.6 µl $MgCl_2$ (50 mM), 1.6 µl of each deoxynucleotide triphosphates (dNTP, 2.5 mM; all from Bioline, Luckenwalde, Germany), 1.0 µl of each pre-primer (5 pmol) and 4 µl of the ligation product with the following temperature profile: 5 min initial denaturation at 94 °C, 20 cycles of 20 s denaturation at 94 °C, 30 s annealing at 56 °C and 120 s elongation at 72 °C.

The pre-amplification product was diluted tenfold with sterile demineralized water. Selective amplification was carried out in a 20-µl volume containing 0.1 µl BioTaq DNA Polymerase (5 U μl^{-1}), 2.0 µl PCR 10× reaction buffer, 0.6 µl $MgCl_2$ (50 mM), 1.6 µl of each dNTP (2.5 mM; all Bioline), 1.0 µl *Mse*I selective primer (5 pmol), 1.0 µl *Eco*RI selective primer (1 pmol), both fluorescence labelled, as well as 3 µl pre-amplification product with the following temperature profile: 1 min initial denaturation at 95 °C, 10 cycles of 20 s denaturation at 94 °C, 30 s annealing at 65 °C (decreasing by 1 °C per cycle), 120 s elongation at 72 °C, followed by 25 cycles of 20 s denaturation at 94 °C, 30 s annealing at 56 °C and 120 s elongation at 72 °C (increasing by 4 s per cycle). For the selective amplification, 21 different primer combinations were tested on 12 samples for their level of variability within and among species, and five primer combinations were chosen for fingerprinting all samples (Table 2). The five combinations were 5′-*Eco*RI + AAG*FAM-3′/5′-*Mse*I + CTG-3′, 5′-*Eco*RI + ACT*HEX-3′/5′-*Mse*I + CTG-3′, 5′-*Eco*RI + AAG*FAM-3′/5′-*Mse*I + CAA-3′, 5′-*Eco*RI + ACT*HEX-3′/5′-*Mse*I + CAC-3′ and 5′-*Eco*RI + AGC*HEX-3′/5′-*Mse*I + CTG-3′.

The main AFLP amplification products in plates (96-well plates, ABgene) were purified by centrifugation (910***g*** at 4 °C) through Multi Screen 96-well plates (Millipore MSHVN4510, Schwalbach, Germany) on a column of Sephadex® G-50 Superfine powder (GE Healthcare Bio-Science, Uppsala, Sweden), and purified amplification products were analysed using a MegaBACE 1000 sequencer (Amersham Biosciences, Freiburg, Germany).

Data analysis

Polymorphic DNA bands were scored as present (1) or absent (0) for each DNA sample. Smeared and weak bands were excluded by visual inspection. A total of 20 randomly chosen samples were used to test the reproducibility of each of the steps of the AFLP procedure including band scoring.

The three primer combinations used in the AFLP analysis of *P. incana* yielded 516 reliable bands. Of these bands, 375 (72.7%) were polymorphic and were consequently used in the analysis (see Appendix S1 in Supporting Information). The number of polymorphic bands per primer pair of *P. incana* ranged between 50 and 98. The percentage of polymorphic bands (P), and Nei's expected heterozygosity (i.e. genetic diversity, He) were calculated to estimate the level of genetic diversity. An analysis of molecular variance (AMOVA) was used to describe genetic structure and to measure the amount of variation found within and between populations; Φ statistics (analogues of *F* statistics) were extracted and significance levels were tested with 999 permutations for each analysis. Populations examined in the AMOVA procedure were assigned to three different groups based on their geographic origins (Páramo de Papallacta,

Table 2 Analysis of molecular variance (AMOVA) for 237 *Polylepis incana* individuals grouped into 10 (sub)populations and three regions (*P* based on 1000 permutations), and for each life stage grouped separately.

	d.f.	Sum of squares	Variance	% of total	Φ statistics	*P*-value
Adults and seedlings						
Among regions	2	789.82	4.60	16	$\Phi_{CT} = 0.156$	< 0.001
Among populations	7	746.37	3.61	12	$\Phi_{SC} = 0.145$	< 0.001
Within populations	226	4806.13	21.27	72	$\Phi_{ST} = 0.278$	< 0.001
Adults						
Among regions	2	411.52	4.69	18	$\Phi_{CT} = 0.175$	< 0.001
Among populations	7	422.97	3.52	13	$\Phi_{SC} = 0.160$	< 0.001
Within populations	108	2000.58	18.52	69	$\Phi_{ST} = 0.307$	< 0.001
Seedlings						
Among regions	2	445.36	4.50	14	$\Phi_{CT} = 0.143$	< 0.001
Among populations	7	553.26	4.84	15	$\Phi_{SC} = 0.180$	< 0.001
Within populations	108	2376.60	22.00	70	$\Phi_{ST} = 0.298$	< 0.001

Páramo de Guamaní and Mojanda; Fig. 1, Table 1). AMOVA was performed with GenAlEx 6.1 (Peakall & Smouse, 2006). Mantel tests (Mantel, 1967), performed with the package vegan (Oksanen *et al.*, 2009) in R version 2.10.0 (R Development Core Team, 2009), were used to examine whether the matrix of genetic differentiation among populations (pairwise Φ_{ST} values) correlated with the matrix of geographical distances.

To infer ancestry groups within the data set, we performed a Bayesian cluster analysis in the program STRUCTURE v. 2.3.3. (Pritchard *et al.*, 2000) in accordance with the approach described by Falush *et al.* (2007), which is strongly recommended by Bonin *et al.* (2007) for dominant marker analysis. We chose the admixture model with correlated allele frequencies among populations with 10,000 burn-in length periods and 500,000 Markov chain Monte Carlo repetitions. Each run was iterated 10 times, with *K* ranging from 1 to 10. The optimum number of *K* clusters was assessed following inspection of the mean values of $L(K)$, $L'(K)$, $L''(K)$ and ΔK, as suggested by Evanno *et al.* (2005). Barplots were created based on the STRUCTURE output files with DISTRUCT (Rosenberg, 2004).

The effects of both altitude and life stage on genetic diversity (*He*) were tested with analysis of covariance (ANCOVA). We ignored the block structure (stands) in the final model, but tested the residuals for spatial autocorrelation. Moran's *I* indicated that autocorrelation in the lower distance classes had $P > 0.1$, indicating that the small-scale structures did not introduce any spatial independence of effects. We thus proceeded with the ANCOVA model, which was simplified by excluding the interaction term. This had no significant effect on model performance. For descriptive purposes, correlations between measures of genetic diversity and altitude as well as fragment area/populations size were quantified using the Spearman rank correlation coefficient (r_S). Analyses were performed in R version 2.10.0, using the special package ncf.

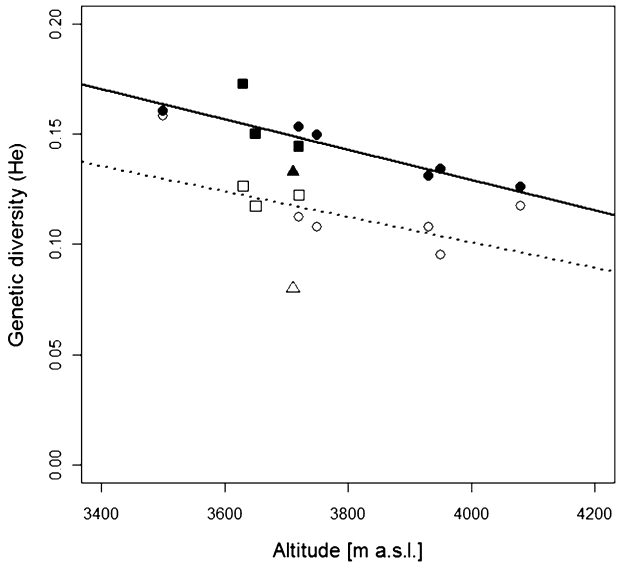

Figure 2 Relationship between altitude (m a.s.l.) and genetic diversity (He) of adults (black figures, solid line) and seedlings (white figures, dotted line) among 10 (sub)populations of *Polylepis incana* in Ecuador. Squares: Páramo de Papallacta; circles: Páramo de Guamaní; triangles: Mojanda.

RESULTS

Measures of within-population diversity yielded moderate values (Table 1). Average percentage values of polymorphism (P) varied between 39.2% and 52.1% in adult populations of *P. incana* and between 26.8% and 50.6% in seedlings. Values of average gene diversity (He) ranged between 0.126 and 0.173 for adults and 0.080 and 0.158 for seedlings (Table 1). According to ANCOVA, the mean diversity of seedlings (He = 0.115) was significantly lower than that of adults (He = 0.145; $P < 0.001$), while elevation had a significant effect on He in both groups ($P < 0.01$). Correlation coefficients between altitude and both measures of genetic diversity were negative for adults (P: $r_S = -0.76$; He: $r_S = -0.77$), the He-value was also negatively correlated in the seedlings (P: $r_S = -0.26$; He: $r_S = -0.54$, Fig. 2). Accordingly, the genetic diversity of subpopulation 7a (forest 7; 3720 m a.s.l.) was lower than that of 7b (3500 m a.s.l.; Table 1, Fig. 2). Using an average genetic diversity value for subpopulations 7a and 7b, which are located in the same forest fragment and therefore counted as a single population in this case, we

found a significant association between He and population size and a marginally significant correlation between He and forest area in the seedlings ($r_S = 0.59$, $P < 0.05$ and $r_S = 0.50$, $P = 0.08$, respectively; $n = 9$). There were no such associations among the adults, nor when using P as a measure for genetic diversity ($P > 0.1$ in all cases).

The AMOVA found that most molecular variance was kept within populations of *P. incana* (adults 69%, seedlings 70%; Table 2). Variance among regions and among populations yielded lower values, which were again similar among life stages (adults 18% and 13%, seedlings 14% and 15%, respectively). The recorded ϕ_{ST} values of 0.307 for the adults and 0.298 for the seedlings provide evidence for strong spatial isolation, particularly when considering the local scale of our study. All 90 pairwise ϕ_{ST} values were highly significant and ranged between 0.074 (2.3 km, subpopulations 7a–7b of forest 7) and 0.397 (19.4 km, population 2–population 9) for the adults, and between 0.042 (2.3 km, subpopulations 7a–7b) and 0.486 (19.8 km, population 7b–population 9) for the seedlings. The results of the Mantel tests confirmed spatial constraints on genetic similarity as geographical and genetic distances among populations were clearly correlated (standardised Mantel statistic; adults $r_M = 0.79$, $n = 45$, $P < 0.001$; seedlings $r_M = 0.75$, $n = 45$, $P = 0.002$).

The results of the Bayesian population structure analysis (Fig. 3) revealed optimum numbers of $K = 3$ clusters for *P. incana* adults and $K = 5$ clusters for the seedlings (see Appendix S2). Clusters of adult plants corresponded to the Papallacta and Guamaní valleys and to Mojanda. For seedlings, the three populations of the Papallacta valley also clustered together, and Mojanda again formed a separate group. The populations of the Guamaní valley separated into two groups: one that contained populations 4, 5, 6 and 8, and another that contained (sub)populations 7 (7a and 7b).

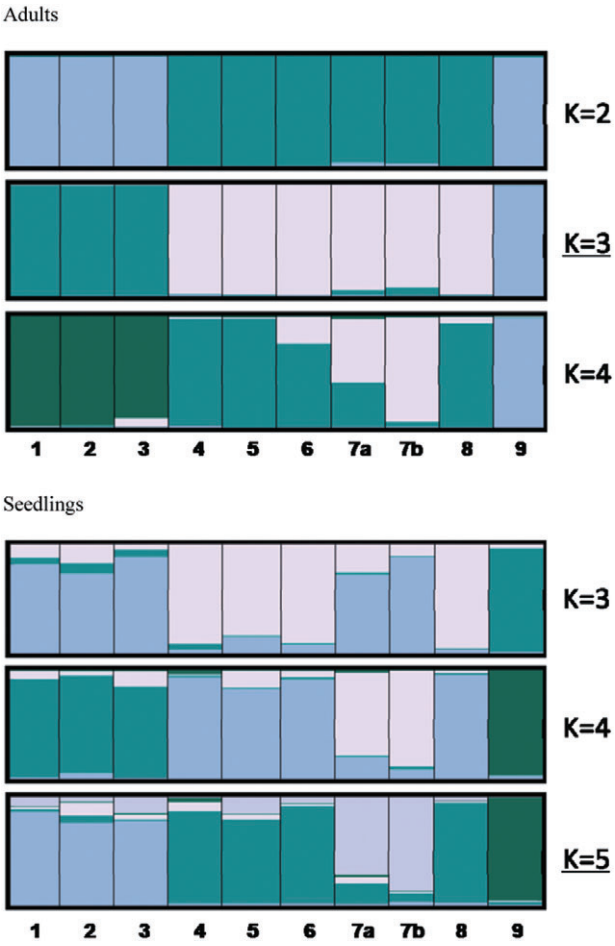

Figure 3 Barplots showing the results of the Bayesian cluster analysis. The colour in each barplot represents the probability of each individual to belong to an admixture group. The underlined cluster numbers $K = 3$ and $K = 5$ were found to be the optimum one for adults and seedlings, respectively. Populations are ordered geographically from left to right: Páramo de Papallacta (1, 2, 3), Páramo de Guamaní (4, 5, 6, 7a, 7b, 8), Mojanda (9).

DISCUSSION

Effects of altitude

Genetic diversity was highly negatively correlated with altitude in both adults and seedlings of *P. incana*. A decrease of genetic diversity towards higher altitudes may be explained by founder events and genetic bottlenecks accompanying historical range expansions at the upper distribution limit, presumably associated with warmer phases (Newton *et al.*, 1999; López *et al.*, 2010). Hence, our data are in accordance with those of Di Pasquale *et al.* (2008), suggesting that common Ecuadorian tree line species shifted to their present position in the latter part of the Holocene. Through soil charcoal analysis, they found the postglacial recolonization of high-altitude sites to be an unexpectedly slow process due to the impact of recurrent fires. An upward migration during Holocene warming periods was also recently presumed for two *Podocarpus* species in temperate Argentina (Quiroga & Premoli, 2007, 2010). In a genetic study of the southernmost *Polylepis* species (*Polylepis australis* in Argentina; I. Hensen *et al.*, pers. comm.), the population at the highest elevation was also found to have the lowest genetic diversity. In accordance, a significant negative correlation between the mean number of alleles per polymorphic locus and average elevation was recorded for *Polylepis pauta* in Ecuador (Aragundi *et al.*, 2011). Central and northern Andean pollen records pointing to the presence of cold-adapted tree genera such as *Podocarpus*, *Weinmannia* and *Alnus* at much lower elevations than today during the last glacial cycle have also been reported by Colinvaux *et al.* (1996) and Behling *et al.* (1999). In accordance, the first fossil record of *Polylepis* type pollen covering multiple glacial–interglacial cycles in the central Andes confirmed several vertical migration cycles of *Polylepis* species in response to past climate change events (Gosling *et al.*, 2009).

Another explanation for diminished genetic diversity at higher altitudes could be a lack of sexual recruitment as a result of the short duration of the growing season and low temperatures (Premoli, 2003; Ohsawa & Ide, 2008), or due to the environmentally associated increasing frequency of clonal

reproduction (Williams & Arnold, 2001; Ohsawa & Ide, 2008; Aragundi *et al.*, 2011). A previous study indeed revealed a reduction in flower numbers and seedling recruitment towards our uppermost *P. incana* forests, which was attributed to environmental constraints (Cierjacks *et al.*, 2008a). Furthermore, *P. australis* shows decreasing seed productivity and seed mass with increasing elevation (Marcora *et al.*, 2008), and some *Polylepis* species show more clonal reproduction at higher elevations (see Hertel & Wesche, 2008 for *Polylepis pepei* in Bolivia; and Cierjacks *et al.*, 2007 for *P. incana* in central Ecuador). The fact that altitudinal trends of gene diversity were largely similar in adults and seedlings implies that filters operating at the reproduction stage are similar along the vertical gradient.

Effects of fragmentation

Our study revealed that fragmentation has severe genetic effects on one of the world's highest tropical mountain forests. Our data are remarkable in that the observed patterns reflect not only recent (seedlings) but also historical (adults) genetic isolation, which was unexpectedly strong given the fact that *P. incana* is wind-pollinated and that distances between forest stands were comparatively short.

Our data are in line with other studies revealing significant impacts of fragmentation on the genetic diversity of tropical tree species (see Lowe *et al.*, 2005, for a review). However, most genetic studies that have included seedlings and adults used insect-pollinated and outcrossing species from the tropical lowlands – which usually grow within stands with high tree species diversity – and data for wind-pollinated species are both rare and usually show limited effects of fragmentation (e.g. Schmidt-Lebuhn *et al.*, 2007; I. Hensen, unpublished data). However, our data agree with the findings of Jump & Peñuelas (2006) and Dubreuil *et al.* (2010), who revealed negative genetic consequences of chronic habitat fragmentation on genetic diversity in the wind-pollinated *Fagus sylvatica* and *Taxus baccata* in the relatively species-poor temperate forests of the Montseny Mountains in Spain. In both studies, the authors used microsatellites and were able to demonstrate significant inbreeding depression, isolation by distance and genetic bottlenecks for the smaller forest fragments, which we assume might act upon *P. incana* as well.

Another striking result from our study is that genetic diversity of *P. incana* seedlings was significantly lower than that of the adults, probably reflecting the ongoing effects of fragmentation processes in terms of population size reduction and increasing isolation. Remarkably, our study populations, which contained between 200 and 81,700 adults, are orders of magnitude larger than those of, for example, *T. baccata* in the study of Dubreuil *et al.* (2010; 30 to 160 individuals). In addition, numbers of adults and seedlings were lowest in the smallest and most isolated of our study populations (population 9 in Mojanda), where the difference between adult and seedling values was the highest of all sampled pairs (Table 1). A similar finding of (slightly) lower seedling diversity compared with adults was recently reported by Julio *et al.* (2008) for two *P. australis* populations in subtropical central Argentina. Our data on seedlings are also in accordance with the common assumption of a relationship between genetic diversity and population size (Leimu *et al.*, 2006), which to date has rarely been confirmed for wind-pollinated tree species. However, adult trees did not exhibit a similar pattern, which again points to ongoing effects of recent fragmentation on the genetic population structure of *P. incana*.

Although the distribution of genetic diversity analysed in this study is in line with several studies that found that wind-pollinated tree species such as *P. incana* have a higher percentage of genetic variability within, rather than among, populations (Nybom & Bartish, 2000; Hamrick, 2004), our findings oppose the general view of low genetic differentiation among populations of wind-pollinated tree species (Hamrick *et al.*, 1992; Kramer *et al.*, 2008). The fixation indices (ϕ_{ST} values) of 0.307 for *P. incana* adults and 0.298 for the seedlings provide evidence for strong spatial isolation, particularly in relation to the small spatial scale of our study. In accordance, our Bayesian cluster analysis revealed that the populations of the Papallacta and Guamaní valleys, as well as that of Mojanda, formed three clearly distinct gene pools; with four distinct pools being detected for seedlings. In contrast, ϕ_{ST} values for *P. pauta* in the humid eastern part of our study region (A. Cierjacks, unpublished data) were 0.126 for adults and 0.283 for seedlings, which points to a different scenario involving the effects of more recent fragmentation (Aragundi *et al.*, 2011). Here, gene flow was historically slightly less hampered through fragmentation or through particular landscape structures. This may reflect the history of human colonization in the central Andes, where drier intra-montane valleys with their benign conditions for agriculture were better suited for the formation of human population centres (Fjeldså, 2007), while the wetter and often steeper outer flanks of the Andes have hosted extensive settlements only over the last few centuries. Thus, *P. incana*, growing in drier and more accessible habitats, may have suffered from fragmentation for a longer period than *P. pauta*.

The strong genetic differentiation we found for *P. incana* populations in the Páramo de Papallacta indicate a general pattern of historically diminished gene flow between forest fragments, even at a restricted spatial scale of only 10 km × 10 km. Thus, the pronounced fragmented forest structure that has been attributed by various authors to grazing and anthropogenic fires (Kessler, 1995; Cierjacks *et al.*, 2008a) may already have existed for several hundred years or even longer. In accordance, our data provide genetic evidence for two seemingly contrasting views on the fragmentation history of the central Andes, as proposed by Purcell & Brelsford (2004) and Di Pasquale *et al.* (2008), who suggest a pronounced long-term period of fragmentation that has become more severe in recent years due to the increasing human impact. In addition, high-mountain landscape structures influence gene flow as the high-mountain crest between both valleys – although only 4200 m high – seems to act as an efficient genetic barrier, which might explain the observed differences between our study and those for other *Polylepis* species. Our data additionally suggest a possible difference in pollen

movement efficiency between *P. incana* and *P. australis* (Seltmann *et al.*, 2007; I. Hensen *et al.*, pers. comm.), but there is currently only sufficient data available on the latter species.

In summary, our data provide evidence that both historic and recent forest fragmentation have contributed to decreased genetic diversity and increased genetic differentiation for a wind-pollinated tropical alpine tree species within a relatively small area. Our data highlight the detrimental impacts of ongoing habitat fragmentation on high-altitude ecosystems, which are much more severe than expected for wind-pollinated species. Consequently, conservation of high-altitude forest ecosystems should aim in particular at maintaining a high connectivity between remaining forest patches as well as increasing overall forest cover. Altitudinal patterns in genetic diversity clearly show that *P. incana* stands did not simply respond positively to historical climate fluctuations. Responses to ongoing climate change may therefore be similarly slow and associated with declines in genetic diversity. As such, simple scenarios describing general range expansion should be modified. We conclude that tropical mountain forests in highly structured habitats may require specific restoration measures and should be cautiously managed to maintain long-term population fitness.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 AFLP raw data for 236 *Polylepis incana* individuals.

Appendix S2 Delta *K* (ΔK) plotted against the number of genotype clusters (*K*).

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BIOSKETCHES

Isabell Hensen is a professor of plant ecology at the Institute of Biology, University of Halle, Germany. Her research, as well as research by several co-authors, is focused on population ecology. This work arises from a project studying the population genetics of South American high-mountain tree line species. Author contributions: I.H., A.C. and K.R. designed the study and collected the data, I.H., H.H. and K.W. analysed the data, I.H. led the writing, A.C., M.K., D.R. and K.W. reviewed and improved the manuscript.

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