The potential genetic responses to environmental changes in forest trees: case studies at multiple spatial scales

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I

– Biowissenschaften –

der Martin-Luther-Universität Halle-Wittehberg

vorgelegt

von Frau Peng, Yanling

geb. am 17. Oktober 1987 in Hunan, China

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Halle (Saale), 09.02.2016

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Summary

Genetic diversity of tree populations determines their adaptability to environmental changes, and is thus essential for the long-term sustainability of forest ecosystems. Thus, understanding the patterns of genetic diversity and their underlying ecological and evolutionary processes are essential for evaluating and predicting the present and future adaptive potential of tree species. In my doctoral thesis, four case studies were undertaken at multiple spatial scales to elucidate the role of different factors such as clonal propagation, elevational gradients, human disturbance and Pleistocene climatic oscillations on the genetic diversity and structure, as well as on the diversification of trees.

The first study examined the impact of clonal propagation on genetic diversity and fine scale genetic structure (FSGS) of the treeline species *Polylepis reticulata* in Ecuador. Lower genetic diversity was detected for *P. reticulata* than has been observed for other *Polylepis* species. Significantly stronger FSGS was detected at the ramet level than the genet level for *P. reticulata* within a spatial distance of 3 m, analyses at the genet level revealed restricted gene dispersal. The study indicates that clonal propagation results in reduced fitness over time due to increased levels of selfing and inbreeding depression. These findings indicate that avoiding the collection of clonal mates for the *ex situ* conservation of this vulnerable species is recommendable.

The second study examined the effects of elevational gradients and human disturbance on the genetic diversity and structure of *Polylepis australis* populations, where individuals exhibit phenological differences in flowering along an elevational gradient. This study tested the hypotheses that phenological differences in flowering arising along elevational gradients lead to reproductive isolation of populations at different elevational zones, which results in elevational genetic structuring, and that forest fragmentation alters elevational genetic structuring. Very low and similar levels of genetic differentiation along elevational gradients were found for adults and saplings. In addition, there was no significant relationship between genetic diversity and elevation. The study indicates that genetic differentiation across elevations was either absent or incipient, and fragmentation does not appear to have affected genetic diversity or differentiation in the studied populations.

The third study tested the effects of Pleistocene climatic oscillations, human disturbance and elevation on genetic diversity and structure of the treeline species *Polylepis tarapacana* in the Andes. High genetic diversity and low genetic differentiation were found for this species, moreover, no significant differences in genetic diversity and differentiation were detected between adults and seedlings. The results indicate that the species most likely migrated up-

and downwards along elevations during Pleistocene climatic oscillations. The findings highlight that Pleistocene climatic oscillations rather than recent human disturbance have influenced genetic diversity in one of the world`s highest treeline species.

The last study examined the effect of Pleistocene climatic oscillations on the diversification of the genus *Abies* in the Qinghai-Tibetan Plateau and Himalayas (QTP). It tested the hypotheses that range expansion caused by climate oscillations in the past promoted morphological radiation in this genus. Strong evidence of range expansion was found by different analyses on a group of 12 fir species in the QTP and Himalayas. This expansion was dated as to the longest and most extensive glaciation in the Pleistocene. The results supported the hypotheses of the range expansion during the Pleistocene for this fir clade; expansion probably drove the morphological radiation of the clade in the QTP and Himalayas although it remains unclear whether the different morphotypes should be acknowledged as independent, reproductively isolated species.

In conclusion, the dissertation detected low genetic diversity for the vulnerable species *P. reticulata,* but high genetic diversity and low genetic differentiation for *P. australis* and *P. tarapacana*. These studies indicate that diminished genetic diversity of *P. reticulate* may cause a loss of fitness and evolutionary capacity to adapt to environmental changes. In contrast, *P. australis* and *P. tarapacana* are more likely to cope with environmental changes in the long term. However, in the face of environmental pressure ranging from timber extraction to global climate change, developing conservation strategies for the long-term survival of these trees is necessary. In addition, the dissertation provides an empirical support for understanding the role of Pleistocene climatic oscillation on the diversification of trees at high elevations.

Zusammenfassung

Die genetische Diversität von Baumpopulationen bestimmt ihre Fähigkeit, sich an veränderte Umweltbedingungen anzupassen und ist daher essentiell für die langfristig nachhaltige Entwicklung von Waldökosystemen. Folglich ist es von großer Bedeutung, die Muster genetischer Diversität und die zu Grunde liegenden ökologischen und evolutionären Prozesse zu verstehen, um das derzeitige und zukünftige adaptive Potential der Populationen zu bestimmen und vorherzusagen. In meiner Doktorarbeit wurden vier Fallstudien auf verschiedenen räumlichen Skalen durchgeführt, um die Rolle zu untersuchen, die verschiedene Faktoren, wie zum Beispiel klonale Fortpflanzung, Höhengradienten, Fragmentierung durch anthropogene Störung und klimatische Veränderungen im Pleistozän, sowohl für die genetische Diversität und Struktur als auch die Diversifizierung von Bäumen spielen.

Die erste Studie untersuchte den Einfluss klonaler Fortpflanzung auf die genetische Diversität und die kleinräumige genetische Struktur (*Fine Scale Genetic Structure*, FSGS) der Art *Polylepis reticulata*, die an den Baumgrenzen Ecuadors vorkommt. Es wurde für *P. reticulata* eine niedrigere genetische Diversität im Vergleich zu anderen *Polylepis*-Arten festgestellt. Desweiteren wurde eine deutlich stärkere FSGS auf Ebene der Rameten als auf Ebene der Geneten innerhalb einer räumlichen Distanz von 3m gefunden. Eine Analyse auf Ebene der Geneten (*Sp*= 0.012, Statistik für die Abnahme der paarweisen Verwandtschaft mit zunehmender Distanz) belegte eine begrenzte Ausbreitung von Genen. Die Studie lässt vermuten, dass klonale Fortpflanzung im Verlauf der Zeit die Fitness durch einen höheren Grad an Selbstbestäubung und Inzuchtdepression, sowie eine dadurch verminderte genetische Diversität, reduziert. Diese Befunde legen nahe, dass es empfehlenswert ist, die Besammlung und Kreuzung von Pflanzen eines Klons für die *ex situ* Erhaltung dieser Art zu vermeiden.

Die zweite Studie beschäftigte sich mit den Effekten von Höhengradienten und anthropogener Störung auf die genetische Diversität und Struktur von Populationen der Art *Polylepis australis*, deren Individuen große phänologische Unterschiede bezüglich der Blüte entlang eines Höhengradienten aufweisen. Diese Studie überprüfte die Hypothese, dass diese phänologischen Unterschiede entlang des Höhengradienten zu einer reproduktiven Isolation von Populationen auf verschiedenen Höhenstufen führen, die wiederum eine starke genetische Strukturierung entlang des Höhengradienten zur Folge hat. Des Weiteren wurde untersucht, ob die Fragmentierung der Wälder die genetische Struktur entlang des Höhengradienten verändert. Sowohl für Jung- als auch für adulte Pflanzen wurde eine sehr geringe und ähnliche genetische Differenzierung entlang des Höhengradienten festgestellt. Außerdem

wurde keine signifikante Beziehung zwischen der genetischen Diversität und der Höhe nachgewiesen. Aus diesen Ergebnissen lässt sich schließen, dass eine genetische Differenzierung entlang des Höhengradienten entweder nicht vorhanden oder unbedeutend ist und dass Fragmentierung anscheinend keinen Effekt auf die genetische Diversität und Differenzierung der untersuchten Populationen hatte.

Die dritte Studie befasste sich mit Effekten klimatischer Oszillation im Pleistozän, anthropogener Störung und der Höhenlage auf die genetische Diversität der Baumgrenzenart *Polylepis tarapacana* in den Anden. Für diese Art wurde eine hohe genetische Diversität und geringe genetische Differenzierung gefunden, des weiteren bestanden keine signifikante Unterschiede hinsichtlich der genetischen Diversität und Differenzierung zwischen juvenilen und adulten Individuen. Die Ergebnisse lassen vermuten, dass die Art während der klimatischen Oszillation im Pleistozän entlang des Höhengradienten abwärts und aufwärts wanderte. Es kann außerdem hervorgehoben werden, dass die klimatische Oszillation im Pleistozän offensichtlich einen größeren Einfluss auf die genetische Diversität dieser Art an einer der weltweit höchsten Baumgrenzen hatte, als rezente anthropogene Störung.

Die letzte Studie adressierte den Effekt klimatischer Oszillation im Pleistozän auf die Diversifizierung der Gattung *Abies* auf dem Qinghai-Tibet-Plateau (QTP) und im Himalaya. Es wurde die Hypothese getestet, dass Arealerweiterungen, die durch vergangene klimatische Oszillation verursacht wurden, die morphologische Radiation der Gattung bedingten. Es wurden starke Bewiese für Arealerweiterungen basierend auf verschiedenen Analysen mit zwölf Tannenarten auf dem QTP und im Himalaya gefunden. Diese Expansion wurde auf die Periode der längsten und räumlich ausgedehntesten Vereisungen im Pleistozän datiert. Die Ergebnisse unterstützen die Hypothese der Arealerweiterung während des Pleistozäns für diese monophyletische Gruppe der Tannen. Die Arealerweiterung trieb vermutlich die morphologische Radiation der Gruppe auf dem QTP und im Himalaya voran, wobei ungeklärt bleibt, ob die verschiedenen Morphotypen als unabhängige, reproduktiv isolierte Arten zu betrachten sind.

Die wichtigsten Ergebnisse der Dissertation lassen sich wie folgt zusammenfassen: Ich konnte eine niedrige genetische Diversität für die gefährdete Art *P. reticulata*, aber hohe genetische Diversität und geringe Differenzierung für *P. australis* und *P. tarapacana* nachweisen. Diese Studien legen nahe, dass die gefährdete Art *P. reticulata* unter ungünstigen Umweltbedingungen überleben könnte, sich aber lediglich auf kurze Sicht an Umweltveränderungen anpassen könnte. Im Gegensatz dazu ist es für *P. australis* und P: *tarapacana* wahrscheinlicher, dass sie mit dem Umweltwandel zurechtkommen. Allerdings

könnten die globale Klimaerwärmung und extremere Fragmentierung zu niedriger genetischer Diversität und höherer genetischer Differenzierung bei diesen Arten führen. Unter diesen Umständen ist es notwendig, Schutzstrategien für die langfristige Erhaltung der Arten zu entwickeln. Schließlich legte diese Dissertation empirische Befunde vor, die das Verständnis der Rolle klimatischer Oszillation im Pleistozän für die Diversifizierung von Bäumen ich Hochlagen erweitern.

Chapter 1

General introduction

Genetic diversity

Genetic diversity is the raw material for evolution and plays an important role in the survival and adaptability of a species (Booy *et al.* 2000), as well as is a key element for the study of biodiversity, ecosystem functioning, and the consequences of human disturbance on natural system (Reusch *et al.* 2005; Hughes *et al.* 2008; Johnson *et al.* 2010; Romiguier *et al.* 2014). Here, genetic diversity can be defined as the amount of variation observed between DNA sequences from distinct individuals of a given species (see Romiguier *et al.* 2014). Genetic diversity is largely attributed to ongoing evolutionary forces, such as mutation, genetic drift, gene flow, and natural selection. It may appear spatially structured at different scales, such as populations, subpopulations or among neighbouring individuals. Recently, diverse DNA-based molecular techniques have become standard tools for assessing genetic diversity.

Forest harbour most of the earth's terrestrial biodiversty, extensively contribute to biogeochemical cycles, and provide countless ecosystem services, including water quality control, climate regulation and carbon sink (Koskela *et al.* 2013). Trees are the keystone species of forest ecosystems maintaining their structure and function. Between 400 billion (Nadkarni 2008) and 3000 billion (Crowther *et al.* 2015) trees are estimated to exist globally. However, the number of trees lost annually is currently 15 billion because of environmental pressure ranging from timber extraction to global climate change; and globally about 46% fewer trees exist compared to the end of the last ice age, when human civilization emerged (Crowther *et al.* 2015). In this context, understanding the patterns of genetic diversity and their underlying ecological and evolutionary processes is essential for assessing their present and future adaptive potential (Pautasso 2009; Alberto *et al.* 2013; Pauls *et al.* 2013). As in other life forms, genetic diversity and structure of trees is largely attributed to life history traits (Hamrick & Godt 1996; Nybom 2004; Aguinagalde *et al.* 2005), environmental conditions (Byars *et al.* 2009; Shi *et al.* 2011), Pleistocene climatic oscillations (Jaramillo-Correa *et al.* 2009), and human disturbance during the last centuries (Jump & Penuelas 2006; Bacles & Jump 2011).

Factors contributing to the patterns of genetic diversity

Life history traits

Genetic diversity and structure of species is largely influenced by their life history traits, including life form, breeding system, mode of reproduction and type of seed and pollen dispersal (Hamrick & Godt 1996; Nybom 2004; Aguinagalde *et al.* 2005). For example, trees generally maintain more intraspecific variation, but display less variation between populations

than other life forms due to long generation times and low mutation rates, usually outcrossing reproduction with high levels of gene flow, and large effective population sizes (Hamrick 2004; Petit & Hampe 2006). Indeed, outcrossing maintains high genetic diversity and weak genetic structure, by promoting the exchange of genes within and among populations (Muir *et al.* 2004; Nettel *et al.* 2009). In contrast, a new allele from a population of self-fertilizing plant species is unlikely to be exchanged among populations, and thus would be lost when the original population goes extinct (Booy *et al.* 2000). Gene flow is defined as the movement of genes within and between populations, indicating that a high level of gene flow is likely to result in high genetic diversity and weak genetic structure. All factors of life history traits contribute to observed patterns of genetic diversity, however, quantifying the effects of clonal propagation on the genetic diversity and structure is essential for survival of species under harsh environmental conditions due to the potential advantage of this reproductive mode under such conditions.

Growing under the harsh environmental conditions, a high proportion of alpine plant species are characterized by both sexual reproduction and clonal growth (Steinger *et al.* 1996; Young *et al.* 2002; Wesche *et al.* 2006; Petit & Hampe 2006), the latter of which becomes more prevalent with increasing elevation (Crawford 2008; de Witte *et al.* 2012). It is well known that sexual reproduction is likely to enhance the level of gene flow among populations via seed dispersal. However, under harsher environmental conditions, species may increasingly suffer physiological stress leading to reduced sexual reproduction (decreased flower, fruit and seed production) (Dorken & Eckert 2001; Eckert 2002; Lui *et al.* 2005), and resulting in limited seed dispersal. In a harsh environment, clonal propagation has several advantages for species surviving: it allows species to survive over long periods of significant climatic oscillation (Honnay & Bossuyt 2005; de Witte *et al.* 2012), has the competitive advantage of allowing plants to expand in space without giving up previous footholds (Linhart & Gehring 2003; Macek *et al.* 2010), and lets plants produce copies of an adapted genotype without the uncertainties associated with sexual reproduction (Linhart & Gehring 2003). On the one hand, clonal propagation may increase the effective number of alleles and heterozygosity in a population (e.g., Balloux *et al.* 2003). On the other hand, clonal propagation can result in reduced fitness over time due to increased levels of selfing and inbreeding depression in self-compatible species (Honnay & Jacquemyn 2008; Seltmann *et al.* 2009). In addition, clonal propagation can strongly affect fine scale genetic structure (FSGS) either through the expected aggregated distribution of clonal mates with identical genotypes or as a component of spatial dispersal (Gliddon *et al.* 1987).

Elevational gradients

Plant species that occur along wide elevational gradients may exhibit high phenotypic variation (Haider *et al.* 2012) as well as genetic differentiation among populations (Ohsawa & Ide 2008 in review; Shi *et al.* 2011). On the one hand, divergent selection pressures promote the evolution of traits adapted to the local environments, that is, local adaptation (Frei *et al.* 2014), whereas gene flow may counteract local adaptation (Lenormand 2002). On the other hand, phenotypic variation may also be due to phenotypic plasticity, that is, the ability of a genotype to adjust its phenotype to different environments without genetic change (Ghalambor *et al.* 2007). Concerning within-population genetic diversity along elevational gradients, four observed patterns were derived at the review by Ohsawa & Ide (2008): (1) diversity peaks on higher slopes are attributed to various reasons like decreased human disturbance and/or historical downslope range shifts due to climate change, and adaptation (e.g., Gamperle & Schneller 2002), (2) greater diversity at lower elevations is considered a result of bottlenecks occurring throughout upward range expansion (e.g., Quiroga *et al.* 2002), (3) higher diversity at intermediate elevations is thought to result from optimal mid-elevation habitat conditions following the central-marginal hypotheses (e.g., Oyama *et al.* 1993), and (4) constant values of genetic variability all over the gradients are attributed to extensive gene flow (e.g., Truong *et al.* 2007).

Human disturbance

The genetic consequences of forest fragmentation caused by human disturbance in trees, especially regarding gene flow, genetic structure, and inbreeding remain a highly debated topic (Kramer *et al.* 2008; Bacles & Jump 2011). Theoretical predictions of reduced genetic diversity and elevated inbreeding following habitat fragmentation (Young *et al.* 1996) are upheld for a number of wind-pollinated temperate tree species (Sork *et al.* 2002; Jump & Penuelas 2006). However, some recent reviews emphasize that tree populations may be resilent to the effects of habitat fragmentation due to high levels of exsiting genetic diversity within populations and evidence that long-distance dispersal of pollen, even in fragmented habitats, is common in many tree species (Hamrick 2004; Kramer *et al.* 2008). Moreover, some tree species have been shown to exhibit enhanced pollen dispersal in fragmented habitats, attributed to the change in the spatial structure as intervening individuals are lost (Dick 2001; Kamm *et al.* 2010; Lander *et al.* 2010). In addition, the lack of the predicted decrease in genetic diversity found in some studies may simply be due to the long generation

time of trees relative to the timing of fragmentation, so that not enough time has passed for drift to reduce genetic diversity (Hamrick 2004; Kramer *et al.* 2008).

Pleistocene climatic oscillations

Climate oscillations during the Pleistocene have driven cycles of range contraction and expansion, influencing the geographical distribution of species worldwide (Hewitt 2000). These changes in distribution have had significant genetic consequences, influencing both spatial variation in genetic diversity and morphological diversification, which could be traced by phylogeographic studies with single or a group of closely related species, especially when lacking of fossil evidence (Avise 2000, 2009; Hickerson *et al.* 2009). Species at high elevations have probably expanded to lower elevations during cooler periods while being restricted to higher elevations during warmer periods, which results low population divergence and weak phylogeographic structures due to the relatively short (interglacial, ca. 15 000 years) periods of isolation and long (glacial, ca. 100 000 years) periods of expansion (Stewart *et al.* 2010). On the other hand, species may also have survived *in situ*, resulting in pronounced genetic differentiation between populations (Opgenoorth *et al.* 2010). In addition, both refugial isolation and range expansion during these climate changes have been found to have caused morphological divergence and recent speciation in some groups (Schluter 2000; Coyne & Orr 2004; Weir & Schluter 2004; Milá *et al.* 2007). Forest trees are useful models to study the impacts of historical events due to their generally large population sizes and long generation times, which allow them to survive drastic environmental changes and to preserve the genetic imprints of such events for a long period (Hamrick 2004; Petit & Hampe 2006).

Study aims and objectives

In this doctoral thesis, four case studies of tree species were undertaken to elucidate the patterns of genetic diversity and their underlying ecological and evolutionary processes from multiple spatial scales. I tried to explain how the trees respond to environmental change based on these genetic studies addressing the four factors contributing to genetic diversity and structure discussed above: 1) clonal propagation, 2) elevational gradients, 3) human disturbance, and 4) Pleistocene climate change. Their results generate predictions for trees facing environmental pressure. The particular studies of this thesis and the corresponding subjects are summarized in Figure 1.

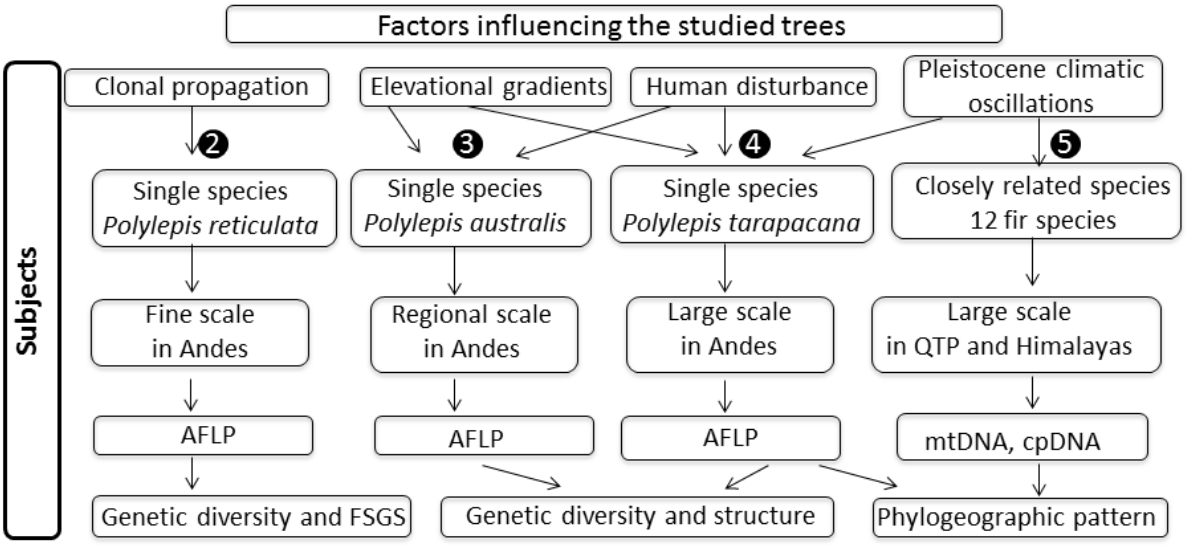

Fig. 1 Overview of the subjects in this thesis. The numbers in the circles represent the chapter numbers.

The first study (Chapter 2) evaluated how life history traits, specifically clonal propagation played a role in the genetic diversity and FSGS in *Polylepis reticulata*, which is found in Ecuador at elevations around 4200 masl. An AFLP study was undertaken to quantify the clonal diversity and FSGS of this vulnerable treeline species. I genotyped 32 and 75 ramets within 4 m × 100 m (coarse scale) and 4 m × 4 m (fine scale) transects of one population respectively. I detected the genetic diversity and the FSGS at the ramet level and the genet level.

The second study (Chapter 3) elucidated the combined factors of elevation and human disturbance on the genetic diversity and structure of *Polylepis australis* in the mountains of Central Argentina. It tested the hypotheses that phenological differences in flowering arising along elevational gradients lead to reproductive isolation of populations at different elevational zones, thereby causing elevational genetic structuring, and that forest fragmentation alters elevational genetic structuring. I assessed the polymorphism of AFLP markers in adults and saplings from two transects covering elevations from 1600 to 2600 masl. I assessed the genetic diversity and differentiation for adults and seedlings, as well as the relationship between genetic diversity and elevation.

The third study (Chapter 3) examined the combined factors of Pleistocene climatic oscillations, human disturbance and elevation on the genetic diversity and structure, as well as phylogeographic pattern of *Polylepis tarapacana*, one of the world's highest treeline species endemic to the central Andes. I compared genetic diversity and structure of 384 adults with

those of 384 seedlings across 32 forest sites spanning a latitudinal gradient of 600 km occurring between 4100 m and 5000 masl using AFLP. I assessed the genetic diversity and differentiation for adults and seedlings, as well as the relationship between genetic diversity and elevation.

The last study (Chapter 4) examined the role of Pleistocene climatic oscillations on the diversification of the genus *Abies* in the Qinghai-Tibetan Plateau (QTP) and Himalayas. It tested the hypotheses that the range expansion during the Pleistocene climatic oscillations probably caused the morphological diversification of this genus. I examined sequence variations in two maternally inherited mtDNA fragments (*nad5-4* and *nad7-1*) and two paternally inherited cpDNA fragments (*trnS-G* and *trnL-F*) for 733 individuals from 75 populations of a group of 12 closely related fir species. I assessed whether range expansion occurred, and if so, dated this expansion.

Study species

The genus Polylepis

The genus *Polylepis* (Rosaceae) consists of ca. 30 tree and shrub species occurring from Venezuela in the north (9ºN) to Argentina in the south (32ºS) (Kessler & Schmidt-Lebuhn 2006; Schmidt-Lebuhn *et al.* 2010). *Polylepis* species are self-compatible (Simpson 1979; Seltmann *et al.* 2009) and their flowers are apetalous, wind-pollinated and proterogynous, and producing single-seeded gravity dispersed nutlets with a low dispersal capacity of up to 10 m (Cierjacks *et al.* 2007; Torres *et al.* 2008). The prevalence of clonal growth at higher altitudes has been reported for some *Polylepis* species (e.g., *Polylepis incana*, Cierjacks *et al.* 2007; and *Polylepis pepei*, Hertel & Wesche 2008;). The longevity of *Polylepis* species could reach up to hundreds of years (Suaréz *et al.* 2008; Solíz *et al.* 2009). Evidence from pollen analyses suggests that the extent of *Polylepis* forests has fluctuated over the past 370 000 years (Gosling *et al.* 2009). Nowadays, the *Polylepis* forests of the Andean highlands are one of South America's most endangered forest ecosystems. In my doctoral thesis, I studied three *Polylepis* species in the South America (Fig. 2; Table 1).

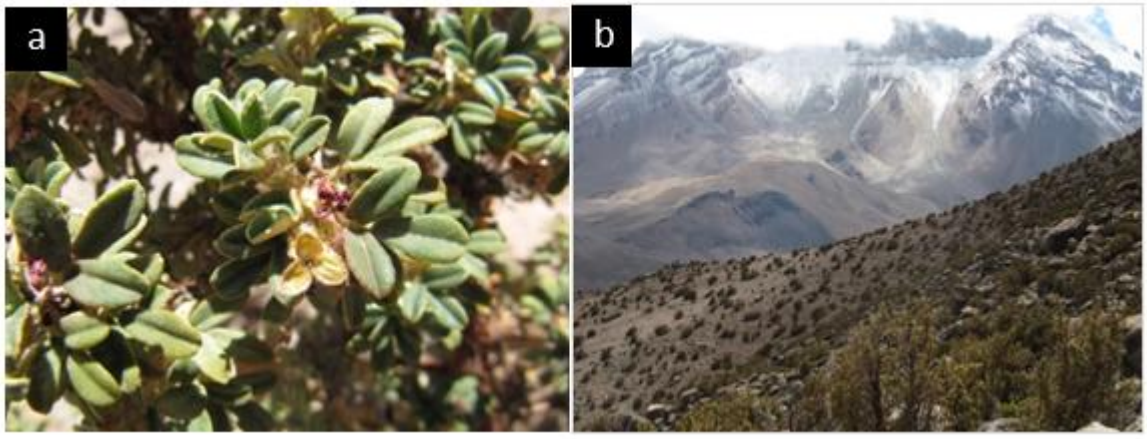

Fig. 2 Illustration of (a) an example of the morphological characteristics of the genus *Polylepis* species, *P. tarapacana*, (b) one forest mainly covered by *Polylepis* species (Photos from Isabell Hensen).

Table 1. The entire natural distribution and ploidy level of the three *Polylepis* species.

Species	Ploidy level	Location	Elevation (m)	References
P. reticulata	2	Ecuador	2850-4300	Romoleroux & Pitman 2004; Schmidt-Lebuhn *et al.* 2010
P. australis	2, 3, 4, 6	Argentina	1000-3000	Kessler & Schmidt-Lebuhn 2006; Schmidt-Lebuhn *et al.* 2010
P. tarapacana	4	Peru, Bolivia, Chile, Argentina	3900-5000	Schmidt-Lebuhn *et al.* 2010; IUCN 2014

The genus Abies

The fir (*Abies*) genus consists of about 50 species which are mainly distributed in the cold temperate and boreal regions of the Northern Hemisphere (Farjon 1990). This genus seems to have undergone only recent diversification in species morphology, given the lack of complete reproductive isolation (Linares 2011) and the long generation time (around 25 years) (Farjon 1990). Of these 50 fir species, 22 species are distributed in China, of which about 70% occur in, and 50% are endemic to, the QTP and Himalayas (Fu *et al.* 1999; Xiang *et al.* 2007). Fir pollen fossils in the QTP can be dated back to the Miocene, but they became more abundant in the Quaternary (Li & Wu 1978; Wang 1992; Xiang *et al.* 2007). The species occurring in these regions have distinctly diverse morphological traits, and they have been tentatively grouped into either three or two different sections (Liu 1971; Farjon & Rushforth 1989). The pollen of these species is wind-dispersed and their seeds are animal-dispersed (Fu *et al.* 1999).

In my doctoral thesis, 12 fir species were studied, they occupied high elevations from 2400 to 4300 masl (Fig 3; Table 2). All of these 12 species are distinguishable in the field, depending on the tree height, the location and the habitat. They can further be identified based on the following characteristics: the length, width, color, and angle of needles, and the presence and absence of stoma on the upper and lower needle surfaces (Fu *et al.* 1999), the 12 species were identified together with Dr. Jianquan Liu, Dr. Bin Tian and Dr. Xinmin Tian.

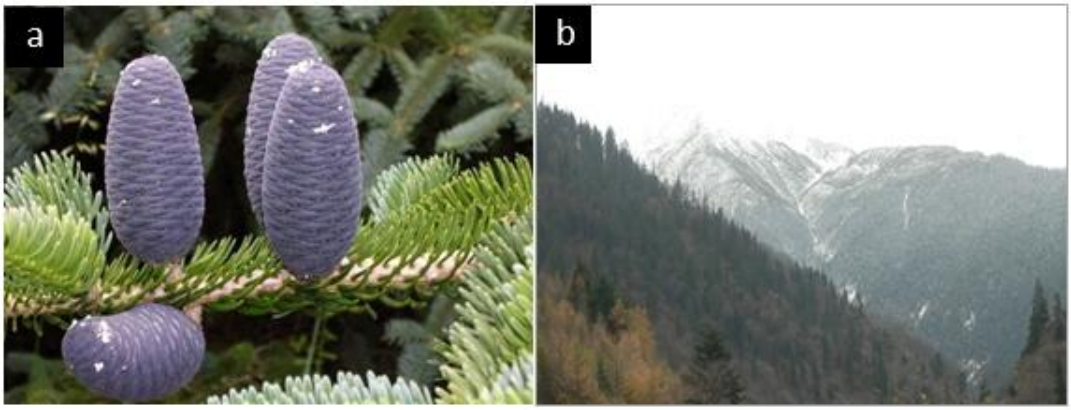

Fig. 3 Illustration of (a) an example of the morphological characteristics of the genus *Abies*, *A. recurvata*, (b) one forest mainly covered by *Abies* species (Photos from Bin Tian).

Table 3. The entire natural distribution and elevations of the 12 fir (*Abies*) species (Fu *et al.* 1999).

Number	Species	Region	Elevations (m)
1	*A. densa*	Xizang	2800-3700
2	*A. spectabilis*	Xizang, Nepal	2600-3800
3	*A. pindrow*	Nepal	2000-3700
4	*A. recurvata*	Gansu, Sichuan	2300-3600
5	*A. squamata*	Gansu, Qinghai, Sichuan, Xizang	3000-4700
6	*A. georgei*	Sichuan, Xizang, Yunnan	2500-4200
7	*A. ernestii*	Gansu, Hubei, Sichuan, Xizang, Yunnan	2600-3900
8	*A. fabri*	Sichuan	1500-4000
9	*A. forrestii*	Sichuan, Xizang, Yunnan	2500-4200
10	*A. nukiangensis*	SichuanYunnan	2500-3100
11	*A. delavayi*	Xizang, Yunnan	3000-4300
12	*A. ferrena*	Sichuan, Yunnan	3300-4000

Study regions

The Andes

The high Andes is believed to be highly sensitive to past and future climate change (Vuille 2008). The Andes is one of the global centres of plant diversity and endemism (Myers *et al.* 2000). The uplift of the Andes started about 20 Mya (Burnham & Graham 1999), but alpine habitats at and above the treeline dated from the Pliocene (Gregory-Wodzicki 2000). Both geological and climatic changes in the Andes have contributed to an increase in the diversity of plants in this high-altitude region (Linder 2008). The genus *Polylepis* (Rosaceae) is represented by several species distributed high elevations in the Andes from Venezuela to Argentina (Schmidt-Lebuhn *et al.* 2010), providing a good opportunity to understand how the highland forest respond to environmental changes.

To understand how trees respond to different factors arising from environmental changes, three of my studies each targeted a different *Polylepis* species from small to large scales (Fig. 4). First, the vulnerable species *P. reticulata* (IUCN 2014) was investigated at a fine scale to detect the effect of clonal propagation. The sampled population was located in the 'Reserva de Producción Faunística Chimborazo' of Ecuador (1.542S, 78.885W) at an elevation of about 4200 masl on SW oriented slopes of 10° - 20° in inclination. The population covers an area of about 30 ha and is composed of several scattered patches varying in densities of from 1 to 15 adult (> 2 m high) trees per 100 m², with ~100 young (< 1 m high) individuals per 10 m² (Fig. S1). Second, *P. australis* was collented over a regional scale in the Córdoba Mountain range of central Argentina (30-32 ºS) to detect the effects of elelevation gradients and human disturbance on populations. The species was sampled along two elevational transects with different disturbance histories covering 1600 to 2600 masl (Fig. S2). Third, the near threatened species *P. tarapacana* (IUCN 2014) was studied at a large scale to detect the effect of Pleistocene climatic oscillations, human disturbance and elevation. It was collected from northwest Argentina, Chile, Bolivia (17-23 ºS). In northern Bolivia and Chile, population sizes exceeded 10 000 adults, while in southern Bolivia and Argentina population sizes were smaller (less than 5 000 individuals).

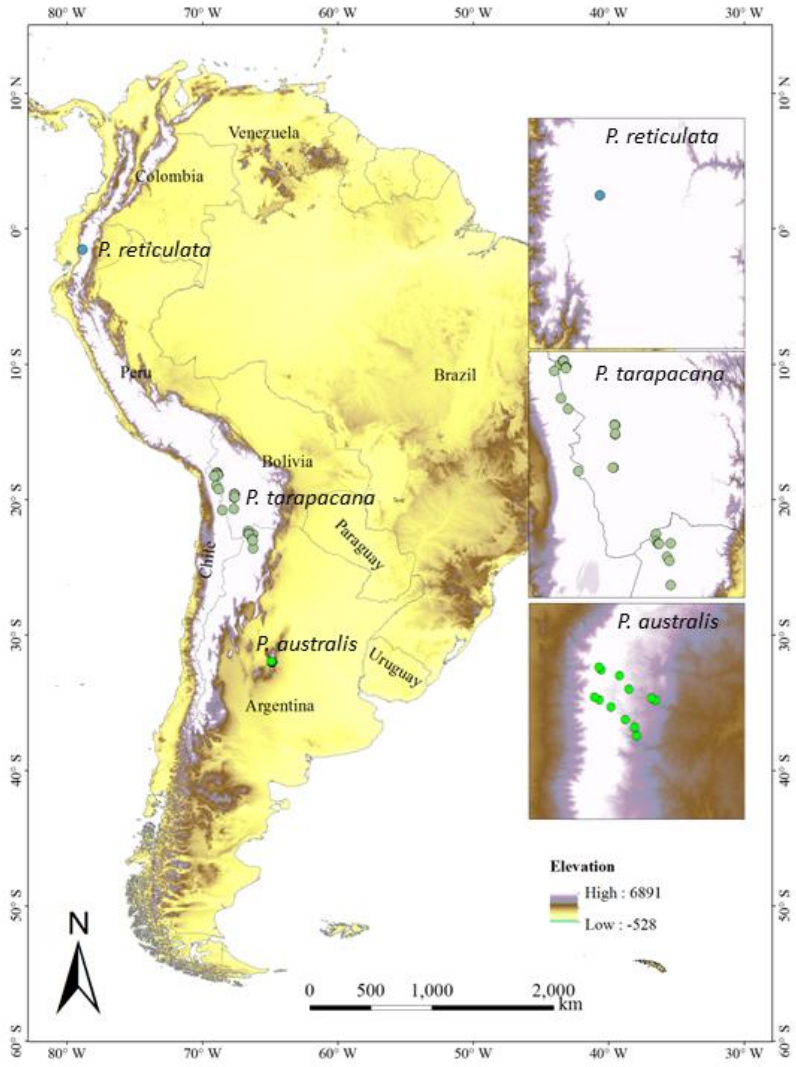

Fig. 4 The general distribution of three studied *Polylepis* species, one plot of *P. reticulata* in Ecuador, 32 plots of *P. tarapacana* in Bolivia, Chile and Argentina, and 12 plots of *P. australis* in Argentina.

Qinghai-Tibetan Plateau (QTP) and Himalayas

The QTP and Himalayas, which comprise the "world's third pole", are believed to be highly sensitive to past and future climatic changes (Zheng 1996). In addition, the extensive uplifts that the QTP has experienced since the early Miocene are likely to have brought about a series of climate alterations (Shi *et al.* 1998). Both geological and climatic changes have contributed to an increase in the diversity of plants in this high-altitude region (Wu 1988), which is known to contain the most diverse alpine flora in the world (Wu & Wu 1996). The eastern part of the QTP and the adjacent regions are listed as one of the world's biodiversity hotspots because of the high level of species richness (Myers *et al.* 2000) Most alpine genera, and those preferring low temperatures, have undergone extensive diversification in this region

(Wang *et al.* 1993; Wu & Wu 1996; Liu *et al.* 2006; Xu *et al.* 2010; Sun *et al.* 2012). The QTP and Himalayas region is not only a diversity hotspot, but also a center of *Abies* richness. In my doctoral thesis, 75 populations from 12 fir species were sampled across the QTP and Himalayas (Fig. 5). The phylogeographic patterns of the genus *Abies* in these regions provides further understanding of the impact of climatic oscillations on the spatial distribution of biodiversity, and thus predicting how high-altitude forest tree species will respond to future climate change.

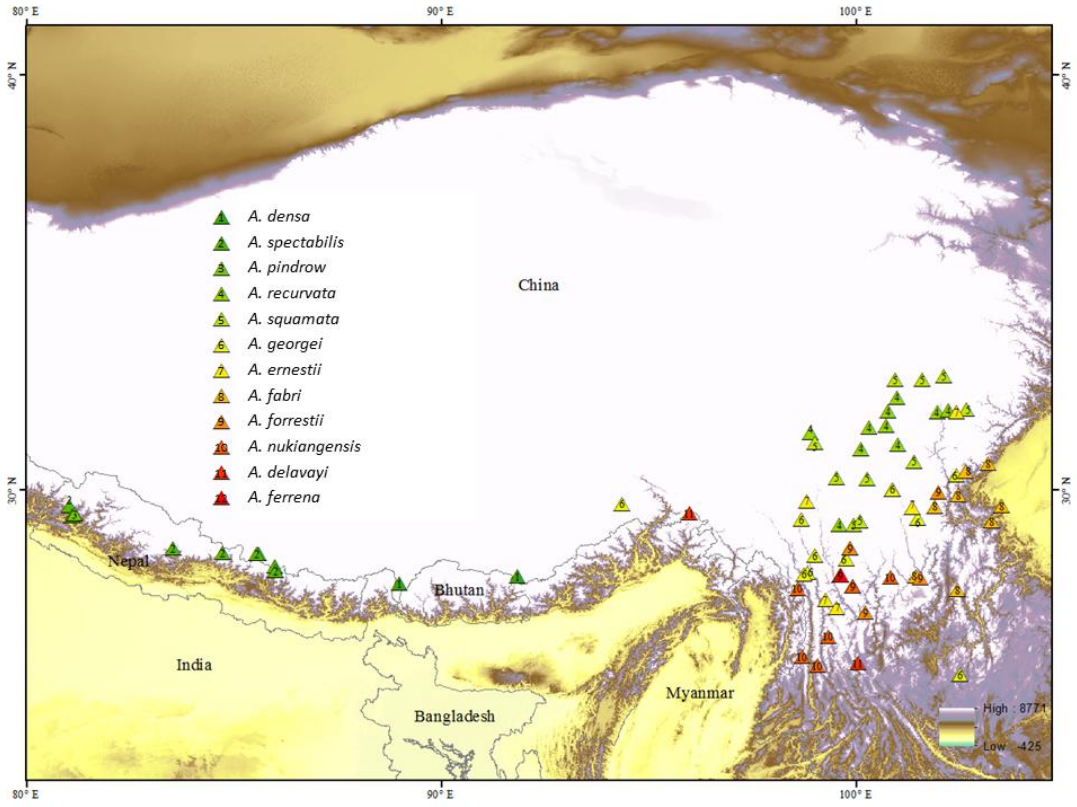

Fig. 5 The general distribution of 75 populations of 12 studied *Abies* species in QTP and Himalayas.

Molecular markers

This doctoral thesis employed Amplified Fragment Length Polymorphism (AFLP; Vos *et al.* 1995) makers to detect the clonal diversity, genetic diversity and genetic structure of the three investigated *Polylepis* species (Fig. 1). AFLP makers provide several advantages over other techniques: it combines the efficiency of PCR with the stability of Restriction Fragment Length Polymorphism (RFLP) analysis, requires small DNA quantities, can simulataneously screen many different DNA regions distributed randomly thoughout the genome and generate many genome wide polymorphic markers with no prior sequence information. AFLP markers have been successfully used to detect genetic diversity and structure, as well as

phylogeographic patterns of other *Polylepis* species with diploid and polyploid cytotypes (Hensen *et al.* 2011, 2012; Gareca *et al.* 2013).

In addition, I employed mitochondrial (mt) and chloroplast (cp) DNA sequence variation to examine phylogeographic patterns in the 12 fir species in the QTP and Himalayas (Fig. 1). As in other conifers, in fir species mtDNA is maternally inherited and dispersed through seeds, while cpDNA is paternally inherited and transmitted by pollen and seeds (Neale & Sederoff 1989; Petit *et al.* 2005). The organelle DNA is usually uniparentally inherited, and genetic polymorphisms therefore arise exclusively from mutation, not from recombination (i.e., through sexual reproduction). As a consequence, organelle DNA markers allow tracing purely uniparental (maternal or paternal) lineages, which are much easier to follow than lineages subject to regular recombination. Sequence variations in these organelle genomes have been widely used to examine both range changes within individual species and interspecific divergence between species that co-occur within local regions (e.g., Liepelt *et al.* 2002; Ziegenhagen *et al.* 2005; Jaramillo-Correa *et al.* 2008; Jiang *et al.* 2011; Wang *et al.* 2011; Peng *et al.* 2012).

Chapter 2

Clonal Diversity and Fine-scale Genetic Structure in a High Andean Tree-line Population

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Biotropica (2015). 47(1): 59-65

ABSTRACT

Clonal propagation becomes more abundant with increasing altitude as environmental conditions worsen. To date, little attention has been paid to the way in which clonal propagation affects genetic diversity and the fine-scale spatial genetic structure (FSGS) of clonal alpine trees. An AFLP study was undertaken to quantify the clonal and genetic diversity and FSGS of the vulnerable treeline species *Polylepis reticulata* in Ecuador. We successfully genotyped 32 and 75 ramets within 4 × 100 m (coarse scale) and 4 × 4 m (fine scale) transects of one population, respectively. Higher genotypic diversity was detected at the coarse scale than at the fine scale, while lower genetic diversity was detected for *P. reticulata* than other *Polylepis spp.* at both scales. Significantly stronger FSGS was detected at the ramet level than the genet level for *P. reticulata* within a spatial distance of 3 m, analyses at the genet level (*Sp* = 0.012, a statistic reflecting declining pairwise kinship with distance) revealed restricted gene dispersal. The studied *P. reticulata* population showed moderate or high FSGS compared to other tropical trees with wind-dispersed pollen and gravity-dispersed seeds, which implies restricted seed dispersal for this population, assuming pollen flow is as extensive as that described for other wind-pollinated tree species. Our results revealed that clonal diversity is a function of both sample size and the spatial scale of the sampling area, as well as clonal propagation have affected FSGS within a spatial distance of 3 m for this species.

Key words: AFLP, clonal diversity, clonal propagation, *Polylepis reticulata*, fine-scale genetic structure, treeline.

ALPINE PLANTS experience harsher environmental conditions at higher altitudes such as lower temperatures and shorter growing seasons (Wesche *et al.* 2006, Reisch *et al.* 2007, Macek *et al.* 2009). Under such conditions, a high proportion of alpine plant species are characterized by both sexual reproduction and clonal growth (Steinger *et al.* 1996, Escaravage *et al.* 1998, Young *et al.* 2002, Wesche *et al.* 2006), the latter of which becomes more prevalent with increasing altitude (Crawford 2008, de Witte *et al.* 2012). This phenomenon may be contributed to the number of advantages associated with clonal propagation. Firstly, clonal propagation allows species to survive over long periods of significant climatic oscillation (Honnay & Bossuyt 2005, de Witte *et al.* 2012). Secondly, clonal propagation has the competitive advantage of allowing plants to expand in space without giving up previous footholds (Linhart & Gehring 2003, Macek *et al.* 2010). In addition, clonal propagation lets plants produce copies of an adapted genotype without the uncertainties associated with sexual reproduction (Linhart & Gehring 2003). On the other hand, clonal growth in plants can result in reduced fitness over time, due to increased levels of selfing and inbreeding depression in self-compatible species and reduced mate availability in self-incompatible species (Honnay & Jacquemyn 2008).

For vulnerable alpine plant species that reproduce both sexually and asexually, it is critical to characterize the effect of clonal propagation on genetic diversity and spatial genetic structure. Fine-scale genetic structure (FSGS) characterizes the spatial distribution of genetic variation within a population and can be quantified using spatial autocorrelation analysis (Reisch *et al.* 2007, Ohsako 2010, Harata *et al.* 2012, Mathiasen & Premoli 2013). Previous studies showed that FSGS commonly resulted from the joint effects of seed and pollen mediated gene flow, as well as the pattern of clonal propagation in the case of clonal plants (Vekemans & Hardy 2004, Chung *et al.* 2006). On one hand, if pollen is dispersed more widely than seeds, the SGS will be influenced more determined by seed dispersal than by pollen dispersal, for example, for the plants with gravity seed-dispersal and wind pollen dispersal (Sebbenn *et al.* 2011, Wang *et al.* 2011). On the other hand, FSGS can be strongly affected by clonal propagation, which can be recognized either through the expected aggregated distribution of clonal mates with identical genotypes or as a component of spatial dispersal (Gliddon *et al.* 1987). When the comparative measures of FSGS are analyzed separately at the ramet and genet level, differences between the two should reflect the contribution of clonal growth to FSGS (Jacquemyn *et al.* 2005, Ruggiero *et al.* 2005). Recently, a growing number of studies have elucidated how clonal propagation affects genetic variation and FSGS among clonal plants at lower altitudes (Steinger *et al.* 1996, Alberto *et al.*

2005, Chung *et al.* 2006, Schleuning *et al.* 2011), as well as non-tree species in the Alps (Pluess & Stoecklin 2004, Reisch *et al.* 2007). However, little attention has been paid to similar studies on alpine clonal trees.

Although clonal propagation has frequently been reported for the high Andean tree genus *Polylepis* (Cierjacks *et al.* 2007, Hertel & Wesche 2008), studies on the quantification of the effects of the clonal propagation on FSGS have yet to be conducted. Here, we present a case study of one population of *P. reticulata* using AFLP to study the effects of clonal propagation on genetic diversity and FSGS of one of the world's highest treeline species. Through the study we aimed to determine: (1) the clonal and genetic diversity at the larger and smaller scales, and (2) the contribution of clonal propagation to the FSGS.

MATERIALS AND METHODS

STUDY SPECIES.—The genus *Polylepis* Ruiz and Pavón (Rosaceae) consists of about 30 wind-pollinated shrub and tree species distributed from southern Venezuela to central Argentina (Kessler & Schmidt-Lebuhn, 2006). The genus is self-compatible (Seltmann *et al.* 2009) and its flowers are apetalous, wind-pollinated and proterogynous, and they have single-seeded gravity dispersed nutlets with a low dispersal capacity of up to 10 m (Cierjacks *et al.* 2007, Torres *et al.* 2008). *Polylepis reticulata* Hieron is a diploid tree (Schmidt-Lebuhn *et al.* 2010) that is endemic to the Andes of Ecuador, grows at altitudes of between 2850 and 4300 m asl, and is classified as vulnerable (Romoleroux & Pitman 2004).

SAMPLING PROCEDURE.—We sampled a population of *P. reticulata* located in the 'Reserva de Producción Faunística Chimborazo' of Ecuador (1.542S, 78.885W) at an elevation of about 4200 m asl on SW oriented slopes of 10° – 20° in gradient. The population covers an area of about 30 ha and is composed of several scattered patches varying in densities of between 1 and 15 adult (> 2 m high) trees per 100 m², with ~100 young (< 1 m high) individuals per 10 m². Conducting a morphological distinction between ramets and genets of the species in the field was not feasible. As such, to assess the clonal diversity and genetic structure of one of the largest patches (160 x 12 m) we collected leaf samples from one fine transect and one coarse transect, with the fine transect being located within the coarse one (see Supplementary Information Table S1, Fig. 1), and we also observed the number of trees with flowers or fruits in total of 20 trees from October 2008 to November 2009 in the sampled location (see Supplementary Information Table S2). The most recently formed leaves were collected in

2009, and leaf surfaces were thoroughly cleaned with tissue paper before being removed and stored in bags with silica-gel in order to reduce contamination by epiphyllic algae and fungi.

We sampled 36 adult individuals along the coarse transect at hierarchal distances (0, 2, 5, 9, 14, 20, 27, 35, 45, 57, 77 and 100 m), with three samples being collected at each interval (middle, left and right; with ~2 m distance between sampled individuals fulfilling the given parameters). In order to assess the fine-scale genetic structure of *P. reticulata*, a total of 81 young individuals were collected from a 9 × 9 sample grid within a 4 × 4 m matrix starting at the 14 m interval of the coarse transect and continuing at 0.5 m intervals, with the nearest appropriate sapling being sampled (Table S1, Fig. 1). Such hierarchical sampling was performed in order to cover a wide range of geographical distances between sampled individuals while minimizing the total number of samples required.

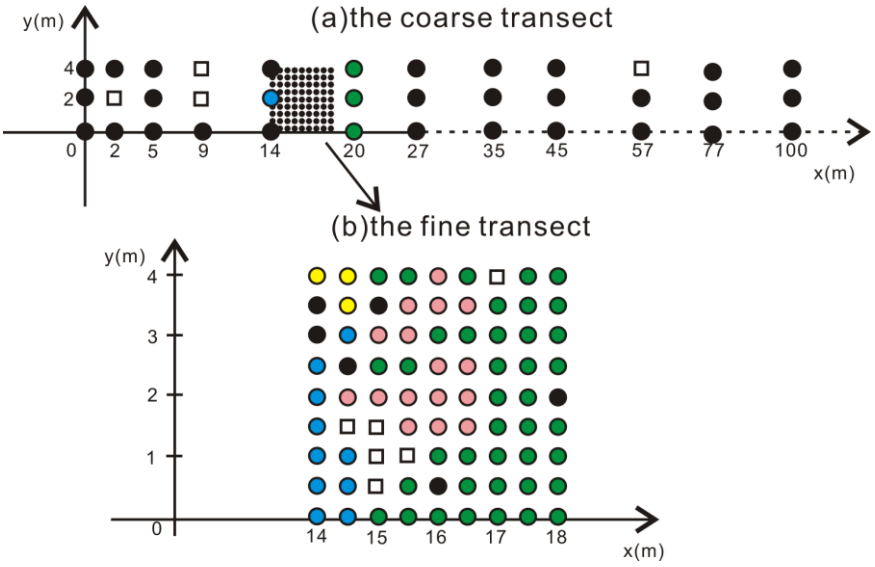

FIGURE 1. The distributions of 38 genets recovered across both transects: a) The distribution of 30 genets (94.0% in total) from the coarse transect; b) The distribution of 10 genets recovered from 75 ramets (13.3% in total). The white square represents the missing samples caused by the PCR problem; the black circles represent different genets; the blue, yellow, pink and green circles represent the remaining four different genets.

DNA EXTRACTION AND AFLP ANALYSES.—Total genomic DNA was extracted from ~20 mg silica-gel-dried leaf material samples using the standard protocol (Doyle & Doyle 1987). All DNA samples were double-digested with the restriction enzymes *Mse*I and *Eco*RI, and the ends of the resulting fragments were ligated to double-stranded adapter oligocleotides (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 containing 1 U of *Mse*I, 5 U of *Eco*RI, 1 U of T4 DNA ligase, 1.1 µl T4 DNA ligase 10 × reaction buffer (all England Biolabs, Frankfurt am Main, Germany), 0.05 mM NaCl, 0.05 mg/ml BSA, 5 pmol of *Eco*RI adaptor, 50 pmol *Mse*I adapter, and 5.0 µl DNA extract. The ligation product was diluted with 39 µl of sterile, demineralised water and preamplified with the primer combination *Eco*RI + A/MseI + C (E01, 5'-GACTGCGTACCAATTC + A-3'/M02, 5'-GATGAGTCCTGAGTAA + C-3'; primer nomenclature following KeyGene, Inc. (2004)). Preamplification was performed in a 20 µl volume containing 0.5 U BioTaq DNA Polymerase, 2.0 µl PCR 10 × reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (all Bioline, Luckenwalde, Germany), 5 pmol of both preamplification primers, 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 preamplification product was diluted 10-fold with sterile demineralised water. Selective amplification was carried out in a 20 µl volume containing 0.5 U BioTaq DNA Polymerase, 2.0 µl PCR 10 × reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (all Bioline, Luckenwalde, Germany), 5 pmol *Mse*I selective primer, 1 pmol fluorescence labeled *Eco*RI selective primer, and 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 4 s per cycle). For the selective amplification, 20 primer combinations were used to scan the polymorphism, with 12 individuals being chosen at random. Four primer combinations were selected to amplify all the samples and consisted of the following: 5'-*Eco*RI + AGC*HEX-3'/5'-*Mse*I + CTG-3', 5'-*Eco*RI + AGC*HEX-3'/5'-*Mse*I + CAT-3', 5'-*Eco*RI + ACT*HEX-3'/5'-*Mse*I + CAA-3', 5'-*Eco*RI + ACT * HEX-3/5'-*Mse*I + CTG -3'.

The main AFLP amplification products in the plates (96-well plates, ABgene, Epsom, United Kingdom) 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 analyzed using a MegaBACE 1000 sequencer (Amersham Biosciences, Freiburg, Germany).

DATA ANALYSIS.—A total of 107 of the 117 sampled individuals were analyzed for the study due to a failed PCR amplification of nine samples (Table 1 and Fig. 1). AFLP raw data were

collected with the MegaBACE Fragment Profiler Version 1.2 (Amersham Biosciences) and scored as present (1) or absent (0) for each sample. Smeared and weak peaks were excluded by visual inspection. A total of 362 reliable fragments were produced in the AFLP analysis of *P. reticulata* using four primer combinations, of which 287 (73.2% in total) were polymorphic and consequently used in the analysis. The number of polymorphic bands per primer of *P. reticulata* ranged from 25 to 95. We performed replicate analyses of 14 samples to determine the reproducibility of AFLP genotyping and obtained an overall error rate of 3.3%.

Table 1. The indices of genetic diversity for ramets and genets, respectively.

Scale	ramets	genets	PD	*D*	Br[10]	He	PLP (%)	Single genets	genets ≥2, <5	genets ≥5
Whole	107	38	0.352	0.811	1.555	0.12	39.0	34	1	3
Coarse	32	30	0.938	0.994	1.595	0.13	43.2	30	1	0
Fine	75	10	0.133	0.662	1.394	0.09	35.5	6	1	3

Abbreviations: PD - clonal diversity; Br[10] - band richness; He - expected heterozygosity; PLP - percentage of polymorphic loci; genets ≥2, <5 – genets contain at least two but less than five individuals; genets ≥5, genets contain at least five individuals.

CLONE IDENTIFICATION.—We used the function "Clones" in the R package "AFLPdat" (Ehrich 2006) to identify clones, *i.e.* genetically identical ramets. All samples were pairwise compared using the statistics software R (version 2.15.0, R Development Core Team 2012). To determine a threshold of pairwise band differences for two genetically differing individuals, a histogram showing the pairwise differences of bands of all individuals within the population was created. The expected band difference BDe (BDe = number of polymorphic loci * error rate) was calculated (Douhovnikoff & Dodd 2003), which produced a threshold of 10 band differences for the clones. We set a limit to the minimal clone size to avoid the inclusion of every single ramet forming clone occupying a clearly much smaller area, as well, we believe we did not cover all ramets of such single ramet clones in our sampling due to the lack of replicates.

CLONAL DIVERSITY AND GENETIC DIVERSITY.—Clonal diversity for both scales was evaluated by the following indices (Ellstrand & Roose 1987, Arnaud-Haond *et al.* 2007): (1) the genotypic diversity PD was calculated as PD = G/N, where G is the number of genets and N is the number of sampled ramets; and (2) a modified version of the Simpson diversity index, $D = 1 - [N_i(N_i - 1)/N(N - 1)]$, where N_i is the number of samples of the *i*th genotype.

Estimates of allele frequencies were computed according to the square root method in AFLP-SURV 1.0 (Vekemans *et al*. 2002). After estimating allele frequencies, statistics on genetic diversity (*i.e*. the percentage of polymorphic loci (PLP) at 5% and Nei's expected heterozygosity *He*) were computed at the genet level in strict accordance with the approach of Lynch & Milligan (1994) and assuming Hardy-Weinberg equilibrium (HW) (*F*is = 0). Calculating heterozygosity from AFLP data requires knowledge of the level of inbreeding in a population. As this is rarely known, a range of possible values is often presented (*e.g*. Kremer *et al*. 2005), and previous studies have assumed HW for other *Polylepis* species on account of their outcrossing nature (Hensen *et al*. 2011, 2012). In addition, band richness (Br) as a rarefaction of 10 genotypes was performed in the software AFLPDiv (http://www.pierroton.inra.fr/genetics/labo/Software/Aflpdiv/). Band richness was defined as the number of phenotypes expected at each locus (*i.e*. each scored AFLP fragment), which can be interpreted as an allelic richness analogue ranging from 1 to 2 (Petit *et al.* 1998, Coart *et al.* 2005).

MANTEL TEST.—We performed a Mantel test between genets within the whole transect using the software GenAlEx 6.5 (Peakall & Smouse 2012). The pairwise, individual-by-individual genetic distance matrix was calculated using the methodology of Huff *et al.* (1993), in which any comparison with the same state yields a value of 0 (both 0 vs 0 comparisons and 1 vs 1 comparisons), while any comparison of different states (0 vs 1 or 1 vs 0) yields a value of 1. Meanwhile, the corresponding geographic distance matrix was produced in Universal Trasverse Mercator (UTM), with data being input in meters. In the case of clones, distances between genets were measured as the distance between corresponding centroid coordinates (average of *x* and *y* coordinates of clone mates). Using a genet's central coordinates for its spatial representation can be justified, as this point is the most parsimonious position of the clone's birthplace.

FINE-SCALE GENETIC STRUCTURE (FSGS).—In order to detect whether the population showed any FSGS, the spatial autocorrelation analyses with kinship coefficients (Hardy 2003) at both the ramet and genet level were quantified with the program SPAGeDi (Hardy & Vekemans 2002). Average kinship coefficients were estimated for the following 15 distance classes: 0.51, 1.1, 1.51, 2.1, 2.51, 3.1, 5.1, 7.51, 10, 20, 30, 40, 50, 70 and 120 m. The average kinship coefficient (F_{ij}) for each distance class was plotted against the spatial distance. Standard errors for the kinship coefficients were estimated using a jackknife procedure over the loci.

FSGS was then quantified by an *Sp* statistic, which represented the rate of decrease of F_{ij} with distance (Vekemans & Hardy 2004); we calculated the '*Sp'* statistic as –b/ (1-F_1), where *b* is the slope of the regression of F_{ij} on the logarithm of spatial distance, and F_1 is the mean F_{ij} between individuals at the first distance interval (Vekemans & Hardy 2004). We tested the significance of *b* against the null hypothesis H_0: $b = 0$ (*i.e.* the overall absence of FSGS) by comparing the observed values with those obtained after performing 1000 random permutations of individuals among positions. *Sp* was estimated based on the genet level. The figure of the spatial autocorrelation was developed using the software SigmaPlot (Systat Software, San Jose, CA). To determine the spatial coordinates, we used sampling grids for the ramets and centroid coordinates for the genets.

RESULTS

CLONAL AND GENETIC DIVERSITY.—A total of 38 genets of *P. reticulata* were detected: 30 within the coarse transect and 10 within the fine transect. Genotypic diversity detected in the coarse transect (PD = 0.938) was clearly higher than that detected in the fine transect (PD = 0.133). The Simpson diversity index (*D*) was 0.994 and 0.662 for coarse and fine transects, respectively. In addition, genetic diversity in terms of percentage of polymorphic loci (PLP), expected heterozygosity (*H*e) and band richness (Br) for the coarse transect and the fine transect were 43.2% and 35.5%, 0.13 and 0.09, and 1.595 and 1.394, respectively (see Table 1).

For both coarse and fine-scale transects, three genets (7.69% in total) consisted of at least ten samples (between 10 and 43, Table 2); one genet (2.56% in total) consisted of three samples, and 34 genets (89.7% in total) consisted of one sample. Clone size ranged from 1.5 to 20 m^2 with an average of 8.8 m^2 (Table 2). Clones consisting of at least five individuals were mostly aggregated (Fig. 1).

Table 2. Size of the analyzed genets of *P. reticulata* consisting of at least five samples. Genets correspond to those in Fig. 1.

Genets	No. of samples	covered area (m^2)
Green	43	20
Pink	16	5
Blue	10	1.5
Mean	23	8.8

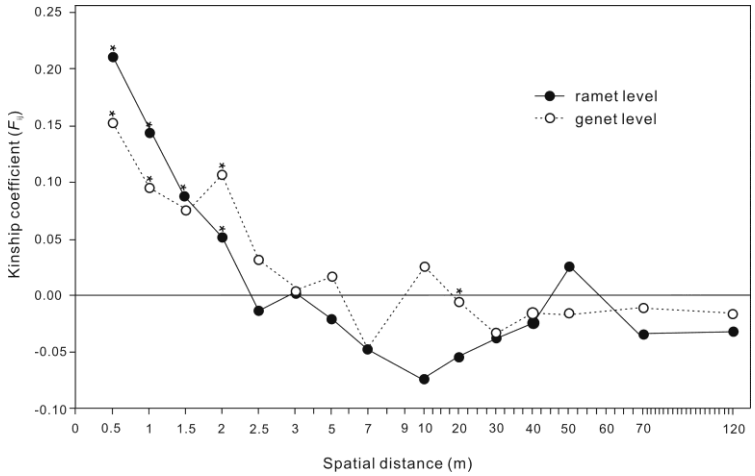

FIGURE 2. Spatial autocorrelation of average kinship coefficient (F_{ij}) over all loci for the whole transect at the ramet level and among-genet level, respectively. '*' indicates kinship coefficient significantly different from zero ($P < 0.05$).

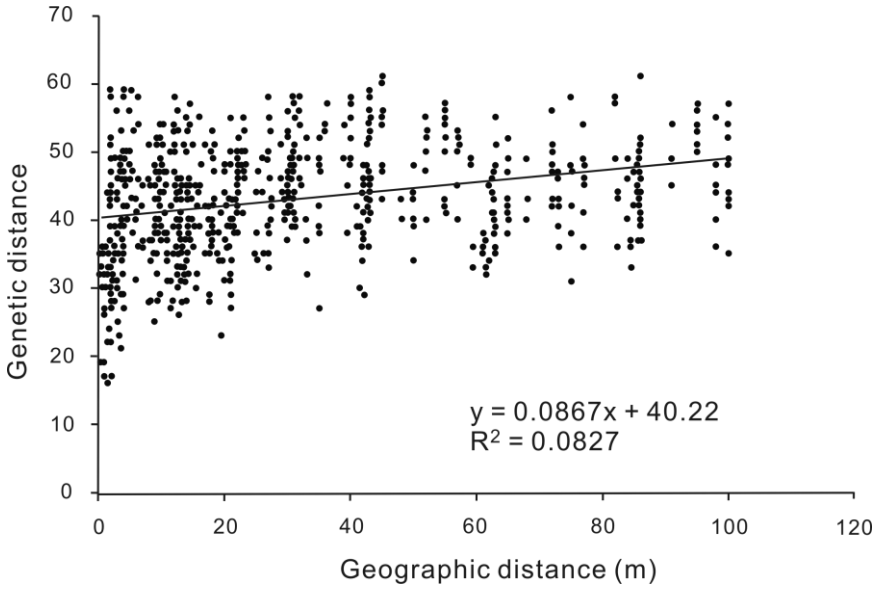

FIGURE 3. Correlation between genetic and geographic distance matrices of studied *P. reticulata* populations along the whole transect (Mantel test).

FSGS AND THE MANTEL TEST.—The spatial autocorrelation analysis revealed a stronger FSGS at the ramet level than at the genet level within the distance interval of 3 m (Fig. 2). At the ramet level, we observed significant positive autocorrelation at the 0.5, 1.0, 1.5 and 2 m distance intervals. Beyond the 3 m interval, no significant F_{ij} values were detected (Fig. 2). At the genet level, we detected significant positive autocorrelation at 0.5, 1.0 and 2 m intervals. Beyond the 2 m interval, we detected only one significant F_{ij} value close to the 20 m distance interval. Based on b = -0.011 and F_1 = 0.0761 for the genets, the value for the *Sp* statistic was

estimated as 0.012 for the whole transect. Finally, we detected a significant relationship between genetic and geographic distance based on data gleaned from the genets ($R = 0.0827$, $P < 0.05$, Fig. 3).

DISCUSSION

In the current study, we found that the clonal diversity of the study population was affected by both sample size and spatial distance, and that clonal propagation influenced the FSGS, but mainly at the finer scale (4x4 m). In comparison with the genetic diversity of other *Polylepis spp*. (Hensen *et al.* 2011, 2012, Quinteros-Casaverde *et al.* 2012, Gareca *et al.* 2013), the genetic diversity values for *P. reticulata* across both transects were low. In addition, the moderate or high *Sp* observed for the genets in this study, compared to that of other tropical trees with wind-dispersed pollen and gravity-dispersed seeds (*e.g.*, Streiff *et al.* 1998, Bizoux *et al.* 2009, Wang *et al.* 2012, Mathiasen & Premoli 2013), indicates limited gene flow by seed between genets, given wind pollen-dispersal is extensive although we did not estimate the pollen gene flow directly.

CLONAL DIVERSITY AND GENETIC DIVERSITY.—Our study revealed that clonal diversity is a function of both sample size and the spatial scale of the sampling area for *P. reticulata*, as was found for other plants (Ruggiero *et al.* 2005, Arnaud-Haond *et al.* 2007). According to our data, the PD value for the coarse transect (0.938) was very high compared to the mean PD of 0.53 for 25 species analyzed with either AFLP, RAPD or ISSR (Honnay & Jacquemyn 2008), while PD on the fine scale was clearly lower (0.133). Our PD value for the coarse transect was also much higher than the mean value reported for the alpine shrubs *Salix hederacea* (0.18, plot 0.3 × 0.3 m, Reisch *et al.* 2007) or *Rhododendron ferrugineum* (0.08, plot 10 × 20 m, Escaravage *et al.* 1998). Given genotypic diversity was positively associated with outcrossing rates at a local scale (that is, among neighboring patches within populations) in some self-compatible species (*e.g.*, *Decodon verticillatus*, Eckert 2000, *Zostera mariana*, Reusch 2001), the contrast level of genotypic diversity between two different transects may be caused by the more frequent occurring of outcrossing among individuals at the coarse transect, due to the extensive wind-pollinated pollen flow.

Despite the high genotypic diversity observed for the coarse transect and low genotypic diversity observed for the fine transect, relatively low genetic diversity was observed for both transects. Our AFLP-derived estimates of the PLP value (43.2%) and genetic diversity (*H*e)

(0.13) at the coarse scale, and the PLP value (35.5%) and *H*e (0.09) at the fine scale detected in *P. reticulata*, are similar to those reported for *P. multijuga* (PLP, 54.9%; *H*e, 0.13; Quinteros-Casaverde *et al.* 2012) and *P. incana* (PLP, 44.8%; *H*e, 0.145; Hensen *et al.* 2012) but much lower than that reported for other *Polylepis spp.* (see Gareca *et al.* 2013). In addition, the *H*e found here was expectedly lower than that reported for other species with similar life histories measured using dominant markers (*H*e = 0.25-0.27, Nybom 2004). The low genetic diversity here seems consistent with several features of *P. reticulata*. Firstly, for the fine transect, the genets of this species contain various numbers of ramets caused by clonal propagation, which can result in reduced fitness over time due to increased levels of selfing and inbreeding depression in self-compatible species (Honnay & Jacquemyn 2008, Seltmann *et al.* 2009). Secondly, for the coarse transect, the population may have experienced genetic drift due to the small population size (*e.g.*, Wesche *et al.* 2006). However, the prolonged lifespan of individuals in *Polylepis* keep this species alive today, as we assume that the longevity of the species could be up to hundreds of years (Suaréz *et al.* 2008, Solíz *et al.* 2009).

CLONAL PROPAGATION CONTRIBUTION TO THE FSGS.—The spatial autocorrelation analysis of kinship coefficients including all pairs of ramets showed a trend of significantly stronger FSGS than that obtained from the analysis considering only pairs between different genets within a 3 m range (Fig. 2), which is attributed to the extensive clonal propagation within this spatial range. Furthermore, it was supported in that our clone sizes ranged from 1.5 to 20 m^2, with an average of 8.8 m^2 for the population (Table 2). Similar results have also been obtained in other fine-scale studies of clonal species (Alberto *et al.* 2005, Chung *et al.* 2006, Ohsako 2010, Schleuning *et al.* 2011), and our study is also in agreement with the prevalence of clonal growth at higher altitudes reported in previous studies on other *Polylepis spp*. (*e.g. Polylepis pepei*, Hertel & Wesche 2008, *Polylepis incana*, Cierjacks *et al.* 2007).

An *Sp* value represents an integrated estimate of both FSGS intensity and extent. It is related to gene dispersal and it is believed to be the most readily comparable estimate of FSGS between sites and species (Vekemans & Hardy 2004). The *Sp* value of *P. reticulata* (*Sp* = 0.012) is high or moderate compared to those reported for some wind-pollinated outcrossing tree species such as *Milicia excels* (maximum *Sp* = 0.006, Bizoux *et al.* 2009), *Castanopsis sclerophylla* (*Sp* = 0.0029-0.0152, Wang *et al.* 2012), low-elevation stands of *Nothofagus pumilio* (*Sp* = 0.003-0.006, Mathiasen & Premoli 2013) or *Quercus robur* (*Sp* = 0.00298, Streiff *et al.* 1998). The highly significant FSGS in our study population is more likely to

have resulted from limited gene-dispersal. It is unknown whether this is due to limited seed or pollen dispersal, but pollen dispersal should be considered more extensive in plants with wind-dispersed pollen (*e.g*. Wang *et al.* 2011), and neighboring populations occurred within the vicinity of this population, *e.g*., one population in the same area *ca* 1km to the north from our plots (with GPS location 1°32'40.92"S, 78°53'02.4"W). The harsh environmental conditions may result in lower seed numbers, as it was very difficult to find fruits for this species in the field (Table S2). Gravity seed-dispersal also causes limited seed dispersal, as seed dispersal of *P. australis* and *P. incana* was found to be restricted to several meters (max. 10 m, Cierjacks *et al.* 2007, Torres *et al.* 2008). In addition, although we found significant correlations between genetic and geographic distance ($P < 0.05$), the mantel correlation (R^2) of 0.0827 is not large (Fig. 3), this pattern may be caused by the extensive pollen dispersal, albeit limited seed dispersal in this population.

An improved understanding of FSGS will enable the development of more effective conservation measures for the species (Chung *et al.* 2006). In *P. reticulata* the FSGS occurred until the 3 m interval, in order to optimize future sampling designs and avoid the collection of clone mates in *ex situ* conservation measures, only individuals occurring > 3 m apart should be collected. Although we sampled only one population in the current study, similar *ex situ* conservation strategies could be provided for other populations of *P. reticulata,* and particularly those that occur in similar alpine habitats.

ACKNOWLEDGEMENTS

The authors thank Petr Sklenář, Ales Soukup and Andrea Kučerová for support in the field, Birgit Mueller for help in the lab, and Walter Durka and Karsten Wesche for support with the data analyses, Sisimac Duchicela for supporting information of flowers and fruits, two anonymous reviewers for attentive reviews. The research was funded by GAAV-A601110702. PM was additionally supported by LM2010009 CzechPolar and JAE-Doc Program co-financed by ESF, and YP was funded by China Scholarship Council (CSC).

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Chapter 3

Low genetic differentiation in *Polylepis australis* along elevational gradients in central Argentina

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Austral Ecology (under review)

Abstract Phenological differences in flowering arising along elevational gradients may lead to reproductive isolation of populations at different elevational zones, and forest fragmentation may either increase or decrease gene flow and potentially alter elevational genetic structuring. For the present study, we aimed to detect whether populations of *Polylepis australis* in the mountains of central Argentina are genetically structured along elevational gradients, and whether any such structure is altered by forest fragmentation. We assessed the polymorphism of AFLP markers in adults and saplings from one conserved and one fragmented forest covering elevations from 1600 to 2600 m asl. Over 98% of variation was found within populations, and we found very low and similar genetic differentiation along elevational gradients for adults and saplings in both the continuous and fragmented mountain forests. In addition, there were no significant relationship between genetic diversity and elevation. However, among-population divergence within any transect range was small, but it was greater than that assessed between different transect ranges. Genetic differentiation across elevations was either absent or incipient, and habitat degradation does not appear to have affected genetic diversity or differentiation in the studied populations. Results therefore imply that for conservation purposes, the study forests may be considered as one genetic unit, and seeds for reforestation may be collected from any location along the two transect routes.

Key words: AFLP, central Argentina, elevational gradients, habitat degradation, *Polylepis australis*

INTRODUCTION

Individuals of plant species distributed across wide elevational gradients can be subject to local adaptation, which can manifest as genetic and phenotypic differentiation (Savolainen *et al.* 2013). For example, elevation can influence the genetic structure of certain plant species characterized by elevation dependent flowering phenologies (Premoli 2003; Alberto *et al.* 2010; Shi *et al.* 2011). Compared to species that adapt to local environmental conditions solely by means of phenotypic plasticity, such genetically mediated adaptation to local conditions may limit plant species' ability to respond to environmental change (Grassein *et al.* 2014; McLean *et al.* 2014). As such, understanding the genetic basis of population adaptation to local environmental conditions is essential to restoration and forestation projects, particularly when determining seed transfer protocols (Potter & Hargrove 2012).

Genetic differentiation among tree populations is most likely altered by forest fragmentation due to the effects fragmentation has on ecological mechanisms that generate or prevent genetic differentiation (Kramer *et al.* 2008). On the one hand, the geographic isolation of individual trees and small forest fragments produced by habitat loss or degradation can augment pollen dispersion rates as intermediate trees disappear and wind or animal dispersed pollen flows more efficiently (Dick 2001; Kamm *et al.* 2010; Lander *et al.* 2010; Bacles & Jump 2011). On the other hand, where severe geographic isolation leads to genetic isolation, affected populations may exhibit less genetic diversity and greater genetic divergence due to genetic drift (Kramer *et al.* 2008; Moreira *et al.* 2009; Gonzales *et al.* 2010). While there are a few studies that examine the effect of fragmentation on the genetic structure of forest trees and plants (e.g. Bacles & Jump 2011) and at least one that examines both elevational patterns and fragmentation effects on the genetic diversity of forest trees (Hensen *et al.* 2012), to the best of our knowledge, there are no published studies testing whether forest fragmentation influences elevational genetic structuring in plant populations.

For the present study, we tested whether forest fragmentation affects elevational genetic structuring in *Polylepis* (Rosaceae) forests in one of the most endangered mountain forest ecosystems of South America, where the species' conservation and reforestation are a priority (IUCN 2014). *Polylepis australis* Bitt. is the southernmost tree species of the genus and it is endemic to Argentina, where it is distributed across a broad elevational range from 1000 m to 3000 m asl (Kessler & Schmidt-Lebuhn 2006). Its distribution has been substantially reduced in recent decades due to fires and livestock browsing, with most forests being restricted to steep ravines and rocky outcrops (Cingolani *et al.* 2008). Remnants of *P. australis* have been exposed to differing levels of historical human impact, which allows for the comparison of

continuous and fragmented forests (Cingolani *et al.* 2008; Renison *et al.* 2011). The historical and contemporary effects of forest fragmentation may therefore be assessed and compared by evaluating the different lifestages of trees, with genetic variation among adults reflecting pre- or historical degradation effects, and more contemporary effects being observable among seedling or saplings (Hensen *et al.* 2012; Vranckx *et al.* 2012).

Previous studies provide useful information on patterns of genetic diversity in other *Polylepis* species in the Andes (Aragundi *et al.* 2011; Hensen *et al.* 2012; Quinteros-Casaverde *et al.* 2012; Gareca *et al.* 2013) including *P. australis* in Argentina (Julio *et al.* 2008; Hensen *et al.* 2011; Julio *et al.* 2011). Julio *et al.* (2008) developed genetic information for a population in a small degraded forest and a population in a large conserved forest using both RAPD and ISSR procedures. They report that there was significant genetic divergence between the young and adult trees in the fragmented forest and between juveniles of both forests, but little genetic divergence was recorded in adult trees between fragmented and continuous forests, suggesting possible diminished gene flow in recent times due to forest loss. In addition, using ISSR and AFLP markers respectively, Julio *et al.* (2011) and Hensen *et al.* (2011) report very little genetic divergence of older trees between different *P. australis* populations. No prior study has apparently evaluated the genetic basis associated with the differences in flowering phenology for the species, in which the flowering dates range from August at the lowest elevations to November at the highest elevations (DR, personal observations). Here, we present the combined effects of elevation and habitat degradation/fragmentation on genetic differentiation among populations of *P. australis* in central Argentina by detecting AFLP variations in adults and saplings from well-conserved and degraded forests situated within elevational gradients ranging from 1600 to 2600 m asl. We originally hypothesized that: 1) With respect to *P. australis* adults, populations are genetically structured along elevational clines as a result of differences in flowering phenology related to elevation in the species; and 2) Genetic differentiation in fragmented forests is lower in saplings than in adults, as wind-dispersed pollen flows more efficiently following habitat loss or degradation.

METHODS

Species description

Polylepis australis is an evergreen phanerophytic Rosaceae that grows up to 14 m tall (Renison *et al.* 2011). While the species is diploid in its northern range, we carried out our study in its southern range, where the species is mainly tetraploid, with some populations

having individuals with polyploidy levels of 2, 3, and 6 at low frequencies (Kessler *et al.* 2014). It is likely that *P. australis* is primarily an outcrosser, as selfing could be hampered by protogyny (Seltmann *et al.* 2007; Seltmann *et al.* 2009). In addition, the species has high pollen viability and longevity, and effective pollen flow (up to 80 km, Seltmann *et al.* 2009). Fruits are single-seeded nutlets, produced annually and dispersed by wind/gravity up to 6 m (Torres *et al.* 2008; Zimmermann *et al.* 2009).

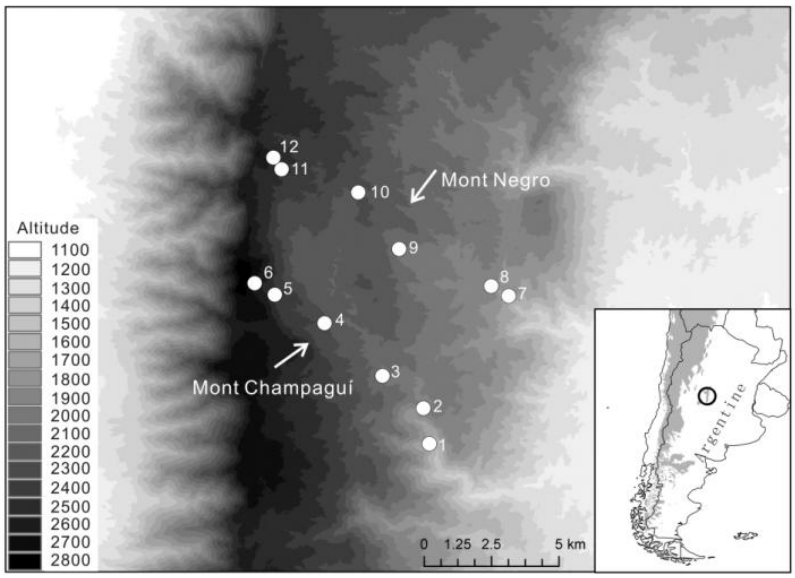

Fig. 1 The distribution of *Polylepis* in one conserved forest - Mont Champaquí (populations 1-6), and one degraded forest - Mont Negro (populations 7-12).

Study area

The Córdoba Mountain range of central Argentina is characterized by a relatively wet, warm season during summer (October to April) and a dry, cold season during winter (May to September). We collected adults (>400 cm) and saplings (<20 cm) from 12 sites (hereafter "populations") distributed every 200 m asl along two elevational transects with different disturbance histories covering 1600 to 2600 m asl (Fig. 1). One transect, leading to Mount Champaquí, followed the “Los Tabaquillos” river through a relatively well preserved forest with a length of 22 km and a width varying from 0.2 to 1.2 km (hereafter the "conserved forest"). Here, *P. australis* represented some 40% of the forest cover and was situated mostly within steep gorges formed by the river and intermingled with a mosaic of rock outcrops and grassland. This area represents one of the most extensive forests fragments found in central Argentina (Cingolani *et al.* 2004). The second transect, leading to Mount Negro, crossed a contiguous yet contrasting site across flatter and higher topographies, which also consisted of

rock outcrops and grassland. In the latter transect, *Polylepis* forests had been extensively burned to support livestock rearing, trees were cut for use as fuel and for construction, and only isolated individuals or small forest patches of up to 50 trees could be found, typically within small inaccessible ridges or stream banks (hereafter the "fragmented forest"). This transect includes one of the most degraded areas of the Central Argentinean mountains (Cingolani *et al.* 2004), which and has remained as such since at least the 1920s (M. Dominguez, personal communication). The geographical distances from the conserved forest to the fragmented forest range between 5 and 12 km. The minimum and maximum distances between the sampled populations were 0.76 km and 8.11 km in the conserved forest, and 0.5 km and 8.3 km in the fragmented forest, respectively.

Sampling

Leaf samples were collected from a total of 144 adults and 144 saplings (height < 20 cm) from six plots per transect measuring approximately 100 × 600 m per plot. Given the size of adults and known growth rate and form analyses, adults were estimated to be older than 100 years (Suarez *et al.* 2008) while saplings were assumed to be less than 15 years old (Renison *et al.* 2015). Within each plot, we randomly sampled 12 adults and 12 saplings with leaves being stored in bags with silica-gel prior to analysis. To minimize the chance of sampling closely related or genetically identical individuals, sampled trees were separated by at least 20 m and care was taken to ensure that plot sizes and distances between sampled trees were similar between the large forest and small forests transects.

DNA extraction and AFLP analysis

Genomic DNA was extracted from approximately 20 mg of silica-dried material using an ATMAB method (Doyle & Doyle 1987). The AFLP method followed Peng *et al.* (2015). We chose four primer pairs that amplified reliably and showed some degree of polymorphism in pre-tests: *Eco*RI AAG - *Mse*I CAT, *Eco*RI AGC - *Mse*I CAT, *Eco*RI AAC - *Mse*I CAA, and *Eco*RI ACT - *Mse*I CAA. Reliable AFLP bands were recovered following the method of Ley & Hardy (2013). We generated an output file for automatic scoring on the Fragment Profile of the MegaBACE (Applied Biosystems) package, which converted peak data into a binary allelic matrix. The output file was prepared for SPAGeDi v1.4 (Hardy & Vekemans 2002) to test for the reproducibility of peaks using broad sense heritability (H^2) and its significance, calculated as F_{ST} of Weir & Cockerham(1984). Peaks with $H^2 > 0.25$ and $P < 0.05$ were considered heritable, and we replicated more than 20% of the total individual for each primer.

The AFLPdat R package (Ehrich 2006) was used to transfer data between the different software packages used.

Data analysis

Given the polyploid nature of our study species, it was not possible to unambiguously estimate allele frequencies. In accordance with Bonin *et al.* (2007), we analyzed AFLP data employing both the band based and fragment-frequency based approaches. We estimated the percentage of polymorphic bands (*PPB*) and Nei's expected heterozygosity (*He*) (Nei & Li 1979; Lynch & Milligan 1994) using a Bayesian method with uniform, prior distribution (Zhivotovsky 1999) in the software AFLPSURV 1.0 (Vekemans *et al.* 2002). We assessed whether genetic diversity (*PPB* and *He*) differed significantly between adults and seedlings with paired *t*-tests using permutations in R 3.1.2 (R Core Team 2014, R-package "broman", Broman& Broman 2014). The dataset was analyzed assuming Hardy-Weinberg equilibrium. We analyzed the effects of elevation on genetic diversity (*He* and *PPB*) for both adults and saplings using ANCOVA in R 3.1.2 (R Core Team 2014). Pairwise F_{ST} values (Weir & Cockerham 1984) were calculated for each population pair using Arlequin 3.5 (Excoffier & Lischer 2010). Significance was evaluated through 1000 permutations.

We employed a Principal Coordinate Analysis (PCoA) to distinguish similar genetic groups of individuals using the package "vegan" (Oksanen *et al.* 2013) in R 3.1.2 (R Core Team 2014). We also used Analysis of Molecular Variance (AMOVA) to describe genetic structure and measure the amount of variation found within and between populations. *F* statistics were extracted and significance levels were tested with 1000 permutations for each analysis, AMOVA was performed with Arlequin 3.5 (Excoffier & Lischer 2010).

To test for isolation by distance (Wright 1943) and any association between pairwise genetic differentiation (F_{ST}) and pairwise geographic distances, we used the Mantel test (Mantel 1967) performed with the package "vegan" (Oksanen *et al.*2013) in R 2.10.0 (R Core Team 2014).

RESULTS

Genetic diversity

Adults and saplings of *P. australis* did not differ in genetic diversity along elevations (ANCOVA: F = 0.308, *P* = 0.58), and genetic diversity did not correlate with elevation (F = 0.061, *P* = 0.081) (Table 1). Genetic diversity in the conserved forest (*PPB* = 80.1, *He* = 0.265 for adults; *PPB* = 79.1, *He* = 0.265 for saplings) was similar to that found in the

fragmented forest (*PPB* = 80.7, *He* = 0.264 for adults; *PPB* = 78.6, *He* = 0.257 for saplings). Adults and seedlings showed no significant difference in genetic diversity in the conserved transect (*PPB*, paired permutation *t*-test: $P = 0.56$; *He*, paired permutation *t*-test: $P = 1$) or in the fragmented transect (*PPB*, paired permutation *t*-test: $P = 0.12$; *He*, paired permutation *t*-test: $P = 0.18$).

Table 1. Genetic diversity estimated as *He* and *PPB* for 12 populations of adults and saplings of *Polylepis australis* respectively.

				Adults			Saplings		
Pop	Lat(S)	Long(W)	Alt(m)	n	*PPB*	*He*	n	*PPB*	*He*
Champaquí Transect					80.1	0.265		79.1	0.265
1	32.046	64.876	1600	11	84.2	0.275	12	78.9	0.257
2	32.034	64.877	1800	10	75.7	0.248	8	73	0.258
3	32.023	64.891	2000	8	76.6	0.268	10	81.8	0.276
4	32.006	64.91	2200	11	79.6	0.264	12	77.2	0.248
5	31.996	64.927	2400	11	82.7	0.264	10	85.4	0.296
6	31.992	64.934	2600	11	81.8	0.27	11	78.5	0.256
Negro Transect					80.7	0.264		78.6	0.257
7	31.997	64.849	1600	11	87.3	0.286	12	83.2	0.275
8	31.993	64.855	1800	11	79.3	0.254	11	79.7	0.262
9	31.981	64.886	2000	11	79.2	0.252	12	77.7	0.249
10	31.962	64.899	2200	11	80.1	0.267	12	78	0.251
11	31.954	64.925	2400	12	78.5	0.255	10	72.8	0.236
12	31.95	64.927	2600	12	80	0.27	12	80.4	0.271

Abbreviations: n - the number of individuals; Lat - Latitude; Long - Longitude; Elev - Elevation.

Genetic differentiation

The AMOVA indicated that while most variation was found within populations (98.6% for adults; 99.1% for saplings), little variation occurred among populations within each forest transect (1.48% for adults; 1.01%, for saplings), with some negative values applying among populations situated between transects (-0.10 for adults and -0.12 for saplings) (Table 2). The negative values between transects indicate that populations from different transects are genetically more closely related than populations within transects. Similarly low levels of genetic differentiation were found between adults in the conserved forest ($F_{ST} = 0.017$) and in the fragmented forest ($F_{ST} = 0.012$). For saplings, genetic differentiation was absent in the conserved forest ($F_{ST} = 0.001$), while it was similar to that of adults in the fragmented forest

(F_{ST} = 0.018). When AMOVA was performed between forests, genetic divergence was F_{CT} = -0.001 for adults and -0.001 for saplings, and thus relatively lower than genetic divergence among populations within each transect (F_{SC} = 0.015 for adults and 0.004 for saplings). Genetic differentiation between populations at different elevations (F_{CT} = 0.005 for adults, F_{CT} = 0.01 for seedlings) was similar to that between populations within the same elevation (F_{SC} = 0.005 for adults, F_{SC} = 0.005). Low significant pairwise F_{ST} was detected among populations for both adults and saplings (Table 3). The PCoA revealed no clusters between the two forests or six elevational belts (Fig. 2) and there were no significant patterns of isolation by distance for the studied species in these regions (Table 4).

Table 2. AMOVA for adults and saplings of *Polylepis australis* calculated from Arlequin ver. 3.5, partitioned by regions and elevations.

Source of variation	d.f.	SS	VC	PV	*F*-statistics
Adults					
Among two forests	1	114.5	-0.11 Va	-0.10	F_{CT} = -0.001
Among populations within forests	10	1214.6	1.57 Vb	1.48	F_{SC} = 0.015***
Within populations	118	12327.1	104.5 Vc	98.62	F_{ST} = 0.014***
Total	129	13656.2	106.0		
Among six elevations	5	634.7	0.512 Va	0.48	F_{CT} = 0.005
Among populations within elevations	6	694.4	1.045 Vb	0.99	F_{SC} = 0.010**
Within populations	118	12327.1	104.47 Vc	98.53	F_{ST} = 0.015***
Total	129	13656.2	106.03		
Among populations within Negro	5	592.9	1.3 Va	1.24	
Within populations	62	6439.2	103.9 Vb	98.76	F_{ST} = 0.012***
Total	67	7032.2	105.2		
Saplings					
Among two forests	1	107.9	-0.13Va	-0.12	F_{CT} = -0.001
Among populations within forests	10	1163.974	1.07 Vb	1.01	F_{SC} = 0.004***
Within populations	120	12560.7	104.7Vc	99.11	F_{ST} = 0.008***
Total	131	13832.6	105.6		
Among sixelevations	5	607.5	0.49 Va	0.46	F_{CT} = 0.005
Among populations within elevations	6	664.3	0.55 Vb	0.52	F_{SC} = 0.005
Within populations	120	12560.7	104.67 Vc	99.01	F_{ST} = 0.010***
Total	131	13832.6	105.71		

Followed by Table S2.

Source of variation	d.f.	SS	VC	PV	*F*-statistics
Saplings					
Among populations within Champaquí	5	537.2	0.1 Va	0.1	
Within populations	57	6060.1	106.3 Vb	99.9	F_{ST} = 0.001***
Total	62	6597.3	106.4		
Among populations within Negro	5	626.8	2.0 Va	1.84	
Within populations	63	6500.6	103.2 Vb	98.16	F_{ST} = 0.018***
Total	68	7127.4	105.1		

Abbreviations: SV - Source of variation; SS - Sum of squares; VC - Variance components; PV - Percentage of variation; *** - P< 0.001.

Table 3. Pairwise F_{ST} between 12 populations. The lower left section shows results for adults, upper right for saplings. Bold text indicates the pairwise F_{ST} value is significant.

	1	2	3	4	5	6	7	8	9	10	11	12
1		0.011	0.005	0.010	**0.028**	0.010	**0.038**	0.014	**0.023**	**0.019**	**0.051**	**0.016**
2	-0.001		-0.026	-0.007	-0.004	-0.010	0.003	-0.023	-0.016	0.003	-0.006	0.014
3	-0.002	-0.007		-0.003	-0.016	-0.012	0.000	-0.025	-0.013	-0.005	0.004	-0.004
4	0.002	0.006	0.016		0.010	-0.004	**0.038**	-0.001	0.002	0.004	0.010	0.012
5	**0.028**	**0.034**	-0.010	**0.058**		0.003	0.007	0.004	0.016	**0.019**	**0.023**	**0.018**
6	0.012	0.009	0.011	0.013	**0.061**		**0.023**	-0.005	-0.001	-0.007	**0.023**	-0.005
7	0.009	0.015	-0.006	**0.025**	0.008	**0.031**		0.006	0.016	**0.033**	**0.043**	**0.033**
8	0.011	-0.003	0.017	0.000	**0.041**	0.005	**0.029**		-0.010	-0.001	0.022	0.005
9	0.005	-0.009	-0.014	0.006	0.021	0.003	0.009	-0.003		0.013	-0.004	**0.022**
10	0.008	0.008	0.020	0.011	**0.050**	0.013	**0.023**	0.001	0.005		**0.036**	0.013
11	0.013	-0.001	-0.008	0.006	0.028	0.016	0.019	0.008	-0.007	0.011		**0.049**
12	0.005	0.017	0.027	0.000	**0.067**	**0.022**	**0.028**	**0.016**	0.015	0.009	0.017	

Table 4. Mantel tests for adults and seedlings of *Polylepis australis*.

	r_M(A)	P (A)	r_M (S)	P(S)
All	-0.0856	0.732	0.1914	0.075
Champaguí	-0.1553	0.72639	0.1865	0.20139
Negro	0.1427	0.27083	0.1072	0.29444

Abbreviations: A - Adults; S - Saplings.

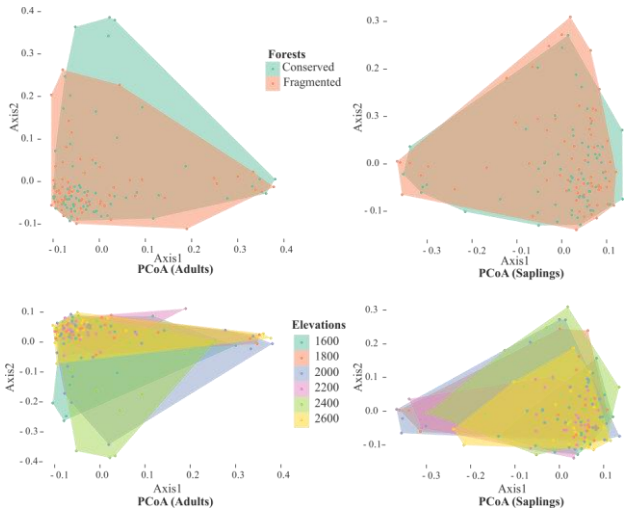

Fig. 2 PCoA from adults and saplings, for each forest transect (upper left and right, each color corresponds to a transect) and for each elevation (lower left and right, each color corresponds to an elevational band).

DISCUSSION

Our study tested the effect of forest fragmentation and flowering phenology along elevational gradients on genetic differentiation in *P. australis* using AFLP markers. Results indicate that there is not enough genetic differentiation to consider populations as being genetically structured along elevational gradients. Inter-population differentiation within the studied forests was higher than that between forests, indicating that phenotypic differentiation in flowering along the elevational gradients may be responsible for higher cross-transect similarity than that within plots. There is no evidence that forest fragmentation has influenced the elevational genetic structure in this study.

The effects of elevation

Genetic diversity (*He* and *PPB*) reached similar levels across the elevational gradients and between degraded and conserved forests for both adults and saplings (Table 1). In accordance, the ANCOVA indicated that there is no significant relationship between genetic diversity and elevation for adults and saplings for both forests. In their respective review, Ohsawa & Ide (2008) reported that genetic diversity of plants along elevational gradients was maintained in 29% of the studies. A likely explanation for this pattern is that extensive pollen dispersal enhances the effective population size in remnants, resulting in the maintenance of the overall gene flow. We rejected seed dispersal as a mechanism maintaining *P. australis* polymorphism, as their wind-dispersed seeds mostly travel distances of less than 6 m (Torres *et al.* 2008; Zimmermann *et al.* 2009). For both forests, genetic differentiation among populations was not

high enough to assume any genetic structuring of populations along elevational gradients (Table 2, Fig. 2). One possible reason is that *P. australis* genetic variation is more prevalent within populations (at least 98% for both forests) than among them. This pattern is consistent with previous studies for *P. australis* (Hensen *et al.* 2011; Julio *et al.* 2011), and it is in accordance with several reviews which showed that wind pollinated tree species have a high proportion of genetic variability within populations and clearly lower diversity among populations (Hamrick *et al.* 2004; Nybom 2004). All these results confirm that extensive gene flow connects populations along the elevational gradient and between the two transects.

However, genetic differentiation among populations along elevational gradients within forests was slightly higher than that between two forests, indicating a disruption of gene flow among populations along elevations. In accordance with similar studies, differences in flowering phenology between populations located at extreme elevations can create temporal restrictions to gene flow (Schuster *et al.* 1989; Premoli 2003; Alberto *et al.* 2010). Elevation might result in reproductive isolation caused by phenological differences, such as different flowering times, which may facilitate adaptation to elevation and lead to neutral genetic differentiation, as shown for other forest trees (Premoli 2003; Alberto *et al.* 2010; Shi *et al.* 2011). However, the limited genetic divergence measured in the present study suggests that populations of *P. australis* along elevational gradients are probably at early stages of genetic differentiation, and they may develop into distinct ecotypes in the future. It is however noted that the neutral markers we used probably in part caused the limited genetic divergence among populations: ALFP are dominant markers, which means that there would be less genetic diversity detected than that using other markers, and less genetic resolution could translate to less differentiation being detected. In addition, neutral markers are not good indicators of selective forces on adaptive traits; however, they are generally considered good indicators of differentiation arising from genetic drift, population isolation or restricted gene flow. Further studies on this species should therefore focus on the genetic basis of local adaptation using more informative markers, such as RAD-sequencing or reciprocal transplant experiments.

The effect of forest fragmentation

The levels of genetic differentiation revealed in our study were very similar between saplings and adults, indicating that habitat fragmentation has not augmented pollen dispersion rates. In addition, habitat fragmentation did not increase genetic differentiation among juveniles, which is not concordant with previous studies on populations of *Polylepis incana* (Hensen *et*

al. 2012) and the only other small population of *P. australis* studied (Julio *et al.* 2008), both of which showed genetic differentiation between juveniles and adult trees. Our results are however consistent with other empirical studies on pollen flow in tree species in fragmented landscapes, all of which demonstrated robust pollen dispersal (White *et al.* 2002; Dick *et al.* 2003; Jha & Dick 2010; Lander *et al.* 2010). Thus, we assume that any loss of genetic connectivity is only expressed in some areas of the Córdoba Mountains, and that it may be a very incipient process. The reasons for the maintenance of genetic connectivity could be due to extensive gene flow, species longevity and/or tetraploidy.

Possible conservation and restoration strategies

Based on the genetic evidence of our study on the joint effects of elevation and forest fragmentation, the two elevational forest transects in this study are not genetically structured along elevational gradients and they are still genetically unaffected by forest fragmentation, indicating that seeds for reforestation purposes could be collected everywhere in these two transects. This scenario probably holds true for other forests in the region, as the study transects represent the largest existing differences in altitudes for the species (Marcora *et al.* 2008). In addition, across the scale of our study, no spatial genetic structure was evident from isolation-by-distance patterns, and all populations were clustered together, indicating that the conserved and degraded forests are probably subject to relicts of an ancestral panmictic forest. This therefore suggests that, in terms of conservation, the two forests may represent one genetic unit.

ACKNOWLEDGEMENTS

We thank Juan Manuel Rodriguez for logistical assistance in the field and Birgit Mueller for help in the lab. Yanling Peng was funded by the China Scholarship Council (CSC, No. 2011618106) and Daniel Renison was funded by CONICET Argentina.

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Chapter 4

Pleistocene climatic oscillations rather than recent human disturbance influence genetic diversity in one of the world`s highest treeline species

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American Journal of Botany (2015). 102(10): 1676-1684.

ABSTRACT

- *Premise of the study*: Biological responses to climatic change usually leave imprints on the genetic diversity and structure of plants. Information on the current genetic diversity and structure of dominant tree species has facilitated our general understanding of phylogeographical patterns.
- *Methods:* Using amplified fragment length polymorphism (AFLPs), we compared genetic diversity and structure of 384 adults of *P. tarapacana* with those of 384 seedlings across 32 forest sites spanning a latitudinal gradient of 600 km occurring between 4,100 m and 5,000 m a.s.l. in *Polylepis tarapacana* (Rosaceae), one of the world's highest treeline species endemic to the central Andes.
- *Key results:* Moderate to high levels of genetic diversity and low genetic differentiation were detected in both adults and seedlings, with levels of genetic diversity and differentiation being almost identical. Four slightly genetically divergent clusters were identified that accorded to differing geographical regions. Genetic diversity decreased from south to north and with increasing precipitation for adults and seedlings, but there was no relationship to elevation.
- *Conclusions:* Our study shows that, unlike other Andean treeline species, recent human activities have not affected the genetic structure of *P. tarapacana*, possibly as a result of the species' inhospitable habitat unsuitable for agriculture. The current genetic pattern of *P. tarapacana* points to a historically more widespread distribution at lower altitudes which allowed considerable gene flow possibly during the glacial periods of the Pleistocene epoch, and also suggests that the northern Argentinean Andes may have served as a refugium for historical populations.

Key words: AFLP, central Andes, elevational gradient, latitudinal gradient, phylogeography, *Polylepis tarapacana*, post-glacial migration

INTRODUCTION

Biological responses to climatic change vary greatly in space and time (Loarie et al., 2009); such responses usually leave imprints on the genetic diversity and structure of plant populations (Hewitt, 2000). Information on the current distributions of the genetic diversity and structure of dominant tree species has facilitated our general understanding of phylogeographical patterns, including post-glacial migration events, from which fossil evidence is lacking. In Europe and North America, genetic diversity of tree species has often been found to decline toward the pole because of post-glacial migration from southern refugia and successive founder events (Hewitt, 2000; Petit et al., 2003).

In South America, the still limited knowledge on tree migration patterns and genetic structure of tropical high-mountain species points to complex scenarios (Quiroga and Premoli, 2007; Pautasso, 2009; Hensen et al., 2011, 2012). In some species, such as *Podocarpus parlatorei*, *Podocarpus nubigena* and *Polylepis australis*, genetic diversity declines with increasing elevation and decreasing latitude (toward the equator; Quiroga and Premoli, 2007, 2010; Hensen et al., 2011). This indicates that these Andean high-mountain tree species migrated toward the equator following historic climate change and expanded to lower elevations during cooler periods while being restricted to higher elevations during warmer periods. According to this scenario, species may exhibit low population divergence and weak phylogeographic structures due to the relatively short (interglacial, ca. 15,000 years) periods of isolation and long (glacial, ca. 100,000 years) periods of expansion (Stewart et al., 2010). For several tree species, the southern distributional areas are assumed to function as long-term refugia (Quiroga and Premoli, 2007). For other species, the results of genetic studies support a scenario of multiple glacial forest refugia in mountain areas, facilitated by the heterogeneous mountain topographies (Premoli et al., 2000; Mathiasen and Premoli, 2009). In such cases, species may have survived *in situ*, resulting in pronounced genetic differentiation between populations (Opgenoorth et al., 2010).

The genus *Polylepis* (Rosaceae) includes about 30 wind-pollinated tree and shrub species endemic to the Andean mountain chain from Argentina and Chile to Venezuela (Kessler and Schmidt-Lebuhn, 2006). As a result of mainly human impacts, *Polylepis* forests represent one of the most endangered ecosystems in the world (IUCN, 2014). Pollen evidence indicates that the current distribution of *Polylepis* forests has been affected by Pleistocene glacial-interglacial cycles (Gosling et al., 2009). The impacts of both Pleistocene climatic changes and more recent human disturbance on the genetic diversity of several *Polylepis* species has been shown by previous genetic studies (Hensen et al., 2011, 2012; Gareca et al., 2013).

The patchy distribution of many *Polylepis* forest stands can be partly explained by the effect of human activities, either directly by timber extraction or indirectly by cattle grazing and associated grassland burning (Kessler, 2002; Renison et al., 2006). Due to recurrent, partly anthropogenic, fires, tropical *Polylepis* forests failed to recolonize high-elevation sites after the Last Glacial Maximum (Di Pasquale et al., 2008; Bush et al., 2015). Additional recent fragmentation through human activity (e.g. timber extraction) might be reflected in the higher genetic diversity observed in adults that germinated before fragmentation and lower diversity in seedlings that developed after fragmentation. This pattern was found for *Polylepis incana* in Ecuador (Hensen et al., 2012), while genetic diversity of *Polylepis subtusalbida* in Bolivia appears not to have been greatly affected by recent human activities (Gareca et al., 2013).

Here, we present a survey of amplified fragment length polymorphism (AFLP) variation within and between populations of *Polylepis tarapacana*, a tetraploid species (Schmidt-Lebuhn et al., 2009) distributed in scattered stands at high elevations in the border regions of Bolivia, Peru, Argentina and Chile. In Bolivia and Argentina, extensive *P. tarapacana* forests have been harvested as a source of charcoal for mining activities since the Spanish conquest. Recent human impact has mainly resulted in habitat degradation, brought about particularly by livestock grazing but also by wood harvesting. The species is listed as *lower risk* or *near threatened* (IUCN, 2014), as such, understanding the genetic consequences of the recent history of the species is of interest to its conservation and sustainable management. Given that the patchy distribution of several *Polylepis* species is the result of human influence (Kessler, 2002; Renison et al., 2015), we expected to find lower genetic diversity in seedlings (< 5 years old) than in adults (> 100 years old). We additionally wanted to explore whether the species survived *in situ* or shifted its elevational and latitudinal range during Pleistocene interglacial-glacial cycles; and suspected that the genetic diversity of this tree species would decrease with increasing elevation and decreasing latitude (toward the equator), as reported already for other Andean tree species (Quiroga and Premoli, 2007, 2010; Hensen et al., 2011).

MATERIALS AND METHODS

Study species—*Polylepis tarapacana* Philippi (Rosaceae) is an evergreen tree species with a mean height of about 3 m, which inhabits the semi-arid Andean highlands from southernmost Peru across western Bolivia to northern Chile and Argentina. The species is one of the world's highest treeline species. It is normally distributed above 3,900 m a.s.l. and can exceptionally reach about 5,000 m on the Sajama Vulcano in Bolivia (IUCN, 2014). The

species is characterized by twisted trunks and branches, compound leaves with leaflets no greater than 1 cm in width, and silvery trichomes on the lower surface (Kessler, 1995). Its flowers are apetalous and wind-pollinated, and fruits are one-seeded gravity-dispersed nutlets with a low dispersal capacity (Cierjacks et al., 2008).

***Species sampling*—**We sampled a total of 384 adults (>2 m high) and 384 seedlings (< 20 cm high) each from 32 forest plots (approx. 200 × 50 m, 12 adults and 12 seedlings per plot). The plots were located within 18 forest remnants covering most of the elevational distribution of the species (4,100 to ~5,000 m) across a latitudinal distance of about 600 km (Table 1, Fig. 1). In northern Bolivia and Chile, population sizes exceeded 10,000 adults, while in southern Bolivia and Argentina population sizes were smaller (less than 5,000 individuals). In seven of the forests, we sampled transects comprising two to four plots over elevational ranges of up to 600 m (Table 1). Sampled individuals were separated by at least 10 m to minimize the chance of sampling closely related individuals. Leaves were stored in bags with silica gel. The distribution of the samples was divided into four geographic regions according to geographic distances and mountain barriers (separating Chile from Bolivia and Argentina): northern Bolivia (National Park Sajama; 144 adults and 144 seedlings from 12 plots distributed across four forests); southern Bolivia (Salar de Uyuni; 84 adults and 84 seedlings; seven plots from three forests); northern Argentina (108 adults and 108 seedlings, from nine plots in seven forests); and northern Chile (48 adults and 48 seedlings, from four forests; Table 1). Climate in the four regions is characterized by a relatively wet, warm season during summer and a dry, cold season during winter. In northern Bolivia, the mean annual precipitation is at about 330 mm (Hoch and Körner, 2005) with a steep decline toward the west into the Atacama desert of Chile, and a less pronounced reduction in precipitation toward the south into northern Argentina, where mean annual precipitation is about 100 mm (Carilla et al., 2013). For each population, mean annual temperature (°C) and precipitation (mm) levels were based on Worldclim 30 sec resolution data (Hijmans et al., 2005). We used the ArcGIS 10 "extract multivalues to points" tool in the Spatial Analyst Extension.

***DNA extraction and AFLP analysis*—**AFLP markers have been successfully used in studies of phylogeographic structures of other *Polylepis* species (Hensen et al., 2011, 2012; Gareca et al., 2013). The AFLP method followed Hensen et al. (2012). We chose five primer pairs which amplified reliably and showed polymorphism in pre-tests: *Eco*RI AGC - *Mse*I CTG, *Eco*RI AGC - *Mse*I CAT, *Eco*RI ACT - *Mse*I CTG, *Eco*RI AAG - *Mse*I CAT, and *Eco*RI

AAG - *Mse*I CTG. To test for reproducibility, we used > 20% replicated individuals (Appendix S1, see online Supplemental Data) and followed the protocol of Ley and Hardy (2013). We generated output files for automatic scoring on the Fragment Profile of the MegaBACE package (Applied Biosystems), which converted peak data into a binary allelic matrix. The output file was prepared for SPAGeDi v1.4 (Hardy and Vekemans, 2002) to test for the reproducibility of peaks using broad sense heritability (H^2) and its significance, calculated as F_{ST}. Peaks with an $H^2 > 0.25$ and $P < 0.05$ were considered heritable for this study (Appendix S1). Among the 768 individuals, readable fingerprints could not be obtained for *Eco*RI AGC - *Mse*I CTG, *Eco*RI AGC - *Mse*I CAT, *Eco*RI ACT - *Mse*I CTG, *Eco*RI AAG - *Mse*I CAT, and *Eco*RI AAG - *Mse*I CTG in 22, 14, 16, 31 and 23 individuals, respectively. These individuals were coded at respective markers as missing data. The AFLPdat R package (Ehrich, 2006) was used to transfer data between the different software packages used.

***Data analyses*—**Given the polyploid nature of our study species, it was not possible to unambiguously estimate allele frequencies. In accordance with Bonin et al. (2007) we analyzed our AFLP data based on both the band-based and fragment-frequency-based approaches.

Genetic diversity at the population level was assessed as the percentage of polymorphic bands (*PPB*) and as Nei's expected heterzygosity (*He*; Nei, 1987) using a Bayesian method with uniform prior distribution (Zhivotovsky, 1999) in the software package AFLPSURV 1.0 (Vekemans et al., 2002). We detected whether genetic diversity (*PPB* and *He*) differs significantly between adults and seedlings with paired *t*-tests with permutations in R 3.1.2 (R Core Team, 2014, R-package "broman", Broman and Broman, 2014). The dataset was analyzed assuming Hardy-Weinberg equilibrium. Pairwise F_{ST} values were calculated for each population pair using Arlequin 3.5 (Excoffier and Lischer, 2010). Significance was evaluated through 1,000 permutations.

We analyzed the relationships between genetic diversity (*He* and *PPB*) and the environmental variables of elevation, latitude, mean annual temperature and mean annual precipitation in interaction with life stage (i.e. seedling vs. adults) using linear mixed effects models with the package "lme4" (Bates et al., 2014) in R 3.1.2 (R Core Team, 2014). For each response variable, we fitted four different ANCOVA models, comprising one of the environmental variables in interaction with life stage, respectively. All models contained the nested random effects of region, forest and population and were fitted with a maximum likelihood approach. The full models were then simplified in a stepwise backward manner by

removing terms that were not significantly based on likelihood ratio (χ^2) tests in order to obtain the minimal adequate models. To check which of the four minimal models explained variation in *He* and *PPB* best, we used AIC (Akaike's "An Information Criterion", Akaike, 1973) adjusted for small sample sizes (AICc, Burnham and Anderson, 2004) in the R package "AICcmodavg" (Mazerolle, 2015). Model analytic plots (Crawley, 2012) confirmed normality of errors and homogeneity of variance for all models with untransformed variables. Moreover, we assessed correlations between pairwises of the four different environmental variables using Pearson's correlation coefficient (r_S).

For the analysis of population structure, a Bayesian model-based approach was implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) for adults and juveniles, respectively. Data were coded as a dominant allele matrix. Due to the tetraploid nature of the species, we handled the genotypes with ambiguous allele copy numbers and analyzed the dataset with the recessive allele option. We ran the analyses under the admixture model with correlated allele frequencies among populations. Ten runs were performed for each number of clusters K = 1-12 using a burn-in and a run length of 100,000 iterations each. The K that best described the data were determined following inspection of the mean values of L(K), L'(K), L''(K) and ΔK (Evanno et al., 2005). The individual ancestry coefficients were calculated by the average pairwise similarity of individual assignments across runs with Clumpp (Jakobsson and Rosenberg, 2007) using the FullSearch method and weighted by the number of individuals in each population; Distruct (Rosenberg, 2004) was used to plot the individual ancestry coefficients.

In addition, we employed a Principal Coordinate Analysis (PCoA) to distinguish similar genetic groups of individuals with the package "vegan" (Oksanen et al., 2013) in R 3.1.2. We also used analysis of molecular variance (AMOVA) to describe genetic structure and to measure the amount of variation found within and between populations; F statistics were extracted and significance levels were tested with 1,000 permutations for each analysis. AMOVA was performed with Arlequin 3.5 (Excoffier and Lischer, 2010). Mantel tests (Mantel, 1967) were used to examine whether genetic distances (pairwise F_{ST} values) correlated with geographic distances using the vegan package (Oksanen et al., 2013). Mantel tests were performed on the entire dataset, each of the four geographic groups, and on the different combination of the geographic groups.

RESULTS

A total of 465 polymorphic markers were obtained from the five primer combinations (Appendix S1). Measures of within-plot diversity yielded high values (Table 1): Average of proportion of polymorphic bands (*PPB*) ranged between 44.7% and 70.5% for *P. tarapacana* adults and between 54.6% and 74.2% for juveniles. Values of average gene diversity (*He*) ranged between 0.17 and 0.21 for both adults and seedlings (Table 1). Adults and seedlings showed no significant difference in genetic diversity *PPB* (paired permutation *t*-test: $P = 0.57$) and *He* (paired permutation *t*-test: $P = 0.90$).

TABLE 1 Geographic data, genetic data and climatic data for the collection sites of adults and seedlings of *Polylepis tarapacana* in the Central Andes.

							Adults			Seedlings		
Pop	F	Lat(ºS)	Long(ºW)	Elev(m)	Mtemp (ºC)	Mpre (mm)	n	*PPB*	*He*	n	*PPB*	*He*
Bolivia North								**64.2**	**0.187**		**64.4**	**0.185**
1	a	18.111	68.962	5000	4.1	342	12	64.9	0.182	12	65.6	0.189
2	a	18.107	68.948	4800	2.2	345	12	63.9	0.181	12	62.2	0.189
3	a	18.105	68.952	4600	2.9	342	12	62.2	0.18	12	63.9	0.187
4	a	18.102	68.958	4400	4	342	12	64.7	0.185	12	65.6	0.187
5	b	18.099	69.032	4415	3.4	339	12	65.2	0.195	12	60.9	0.177
6	c	17.998	68.934	4800	1.8	360	12	65.2	0.192	12	64.3	0.183
7	c	18.007	68.953	4600	2.7	357	12	64.1	0.182	12	66	0.183
8	c	18.012	68.935	4400	3.2	353	12	66.9	0.197	12	63.7	0.178
9	d	18.155	68.863	4920	1.1	342	12	67.7	0.193	12	66	0.194
10	d	18.157	68.866	4800	1.1	342	12	69	0.192	12	64.5	0.19
11	d	18.166	68.872	4600	1.9	338	12	58.7	0.177	12	63.7	0.175
12	d	18.182	68.877	4400	3.5	335	12	58.1	0.189	12	66.7	0.19
Bolivia South								**65**	**0.198**		**65.3**	**0.198**
13	e	20.654	67.700	4557	4	94	12	64.5	0.202	12	71.4	0.201
14	e	20.656	67.706	4345	4.5	95	12	68.6	0.204	12	54.6	0.193
15	f	19.599	67.647	4130	4.6	181	12	63.9	0.203	12	59.1	0.202
16	f	19.584	67.665	4554	4.9	185	12	66.7	0.188	12	64.9	0.212
17	f	19.596	67.666	4351	5.5	188	12	62.2	0.193	12	64.3	0.194
18	g	19.832	67.655	4769	3.1	155	12	69.5	0.199	12	68.4	0.188
19	g	19.812	67.647	4578	3.8	158	12	59.6	0.2	12	74.2	0.194

Followed by TABLE 1

Pop	F	Lat(ºS)	Long (ºW)	Elev (m)	Mtemp (ºC)	Mpre (mm)	Adults			Seedlings		
							n	*PPB*	*He*	n	*PPB*	*He*
Argentina							**67.3**	**0.194**		**67.7**	**0.196**	
20	h	22.534	66.273	4583	4.7	126	12	67.1	0.194	12	70.1	0.197
21	i	23.575	66.273	4466	5.4	116	12	63.9	0.198	12	62.4	0.197
22	j	22.301	66.635	4398	5.4	98	12	68.2	0.192	12	69.7	0.195
23	k	22.478	66.632	4302	6.1	101	12	70.5	0.206	12	72.7	0.202
24	l	22.536	66.571	4588	4.1	104	12	70.1	0.201	12	66.9	0.189
25	l	22.550	66.571	4794	2.6	105	12	69	0.196	12	70.1	0.191
26	l	22.548	66.562	4942	2.5	106	12	63.2	0.178	12	61.7	0.19
27	m	22.871	66.355	4433	5.1	117	12	63.4	0.183	12	66.9	0.194
28	n	22.976	66.304	4644	3.7	120	12	69.9	0.198	12	69	0.207
Chile							**59.4**	**0.185**		**62.4**	**0.186**	
29	o	18.250	69.167	4545	2.1	312	12	59.8	0.176	12	63.2	0.183
30	p	18.935	69.001	4550	1.8	246	12	44.7	0.173	12	63.9	0.19
31	q	19.197	68.817	4209	4	210	12	66.5	0.187	12	63	0.187
32	r	20.751	68.567	4250	4.1	81	12	66.7	0.204	12	59.4	0.184
Total								**64.6**	**0.191**		**65.3**	**0.191**

Abbreviations: Pop, Populations; F, Forest remnant (a-r); n, the number of individuals; Lat, Latitude; Long, Longitude; Elev, Elevation; Mtemp, Mean annual temperature; Mpre, Mean annual precipitation; *PPB*, percentage of polymorphism bands; *He*, Heterozygosity.

According to the ANCOVA, we found that *He* was significantly positively related to latitude ($\chi^2 = 5.98$, df = 1, $P < 0.05$) and mean annual temperature ($\chi^2 = 3.85$, df = 1, $P < 0.05$), and significantly negatively related to mean annual precipitation ($\chi^2 = 8.07$, df = 1, $P < 0.01$), while elevation had no significant effect. None of the environmental variables had a significant effect on *PPB*. In all cases, neither the interaction nor the main effect of life stage was significant. For *He*, mean annual precipitation explained the data best (AICc = -438.0), followed by latitude (AICc = -435.9), mean annual temperature (AICc = -433.8), and elevation (AICc = -432.1). In addition, based on Pearson's correlation coefficient (Fig. 2), we found elevation and latitude to be uncorrelated, but they were both significantly correlated to mean annual temperature (elevation: $r_S = -0.61$, $P < 0.001$; latitude: $r_S = 0.57$, $P < 0.001$) and mean annual precipitation (elevation: $r_S = 0.27$, $P < 0.05$; latitude: $r_S = -0.90$, $P < 0.001$). The two climatic variables, in turn, were negatively correlated ($r_S = -0.61$, $P < 0.001$).

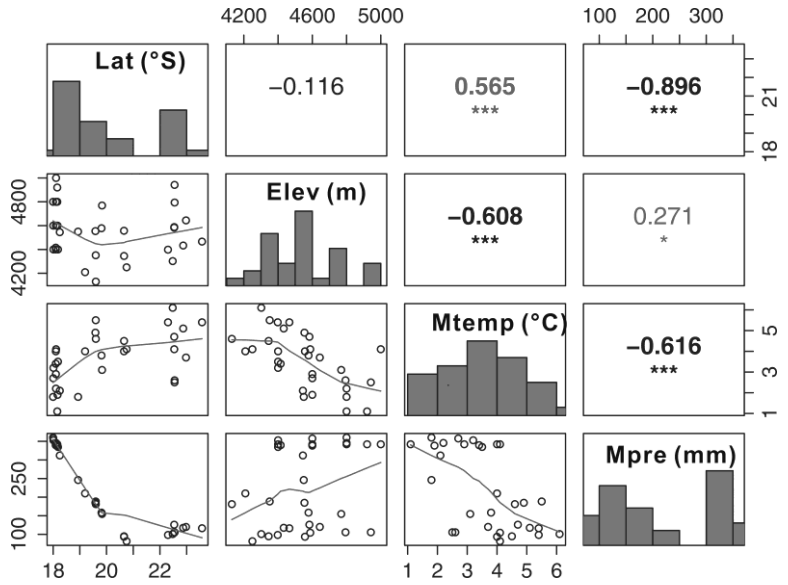

Fig. 2 Pearson's correlation coefficient between pairwise factors. Abbreviations: Lat, Latitude; Elev, Elevation; Mtemp, Mean annual temperature; Mpre, Mean annual precipitation.

The results of the Bayesian structure analysis (Fig. 3) revealed peak values of K = 2 and 4 clusters for *P. tarapacana* adults and K = 2 and 4 clusters for the seedlings (Appendix S2, see online Supplemental Data). PCoA (Fig. 4) also showed four main clusters. Clusters of adult plants corresponded to Bolivia North, Bolivia South, Argentina and Chile, and seedlings produced the same clusters. The overall fixation indices (F_{ST} values) of 0.102 for *P. tarapacana* adults and 0.100 for the seedlings provided evidence of weak spatial isolation (Table 2). The pairwise F_{ST} values were highly significant and ranged between 0.055 (Bolivia North and Argentina) and 0.132 (Bolivia North and Chile) for adults, and 0.054 (Bolivia North and Argentina) and 0.126 (Bolivia North and Chile) for seedlings (Table 3).

The AMOVA found that molecular variance was highest within plots of *P. tarapacana* (adults 89.75%, seedlings 89.96%, Table 2). Variance among four geographic regions and among forest remnants yielded lower values, which were again similar between adults and seedlings (7.11% and 3.14% vs. 6.6% and 3.44%, respectively). The Mantel test indicated isolation by distance across all forest plots for adults (r_M = 0.342) and seedlings (r_M = 0.364; Table 4) and for the Argentinean cluster, but no isolation by distance was detected for the other three clusters.

TABLE 2 AMOVA calculated with Arlequin ver. 3.5.

	Adults			**Seedlings**		
SV	d.f.	PV	*F*-statistic	d.f.	PV	*F*-statistic
All						
Among 4 clusters	3	7.11	F_{CT} = 0.071***	3	6.6	F_{CT} = 0.066***
Among populations within cluster	28	3.14	F_{SC} = 0.034***	28	3.44	F_{SC} = 0.037***
Within populations	324	89.75	F_{ST} = 0.102***	328	89.96	F_{ST} = 0.100***
Total	355			359		
Bolivia North						
Among populations	11	1.54		11	1.12	
Within populations	126	98.46	F_{ST} = 0.015***	129	98.88	F_{ST} = 0.011***
Total	137			140		
Bolivia South						
Among populations	6	4.88		6	5.23	
Within populations	60	95.1	F_{ST} = 0.048***	62	94.77	F_{ST} = 0.052***
Total	66			68		
Argentina						
Among populations	8	4.57		8	6.19	
Within populations	95	95.43	F_{ST} = 0.046***	97	93.81	F_{ST} = 0.062***
Total	103			105		
Chile						
Among populations	3	3.66		3	2.5	
Within populations	43	96.34	F_{ST} = 0.037***	40	97.5	F_{ST} = 0.025***
Total	46			43		

Abbreviations: SV, Source of variation; PV, Percentage of variation; ***, $P< 0.001$.

TABLE 3 Pairwise F_{ST} between the four clusters. The lower left part shows results for adults, while the upper right shows seedlings. Bold text indicates the pairwise F_{ST} value is significant.

	BN	BS	A	C
BN	0	**0.05895**	**0.0539**	**0.12649**
BS	**0.05475**	**0**	**0.05717**	**0.07943**
A	**0.0545**	**0.06093**	**0**	**0.08302**
C	**0.13166**	**0.09554**	**0.09988**	**0**

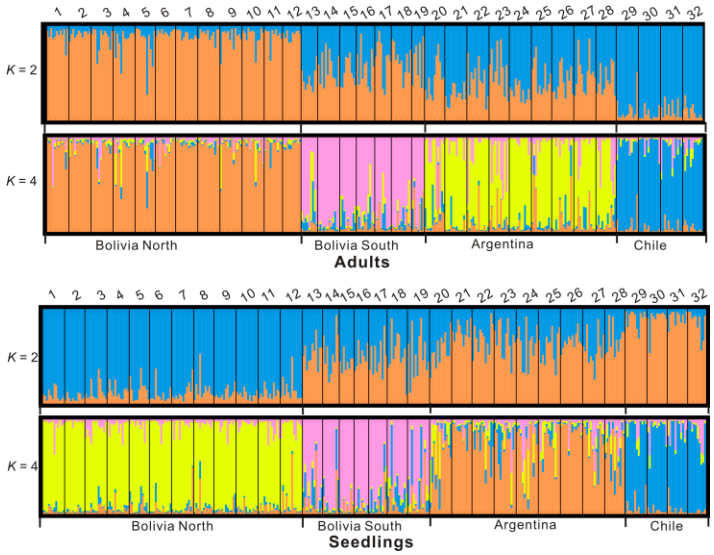

Fig. 3 Bayesian structure analysis of *Polylepis tarapacana* with the STRUCTURE software. Probability of assignment for K = 2 and 4 for adults (above), and K = 2 and 4 for seedlings (below) in 32 populations respectively. Each individual is represented by a single vertical line divided into K colored segments, where K is the number of genetic clusters. Black ticks separate the regions indicated below the figure. Labels above the plots indicate population information, and the labels below the plots provide region information.

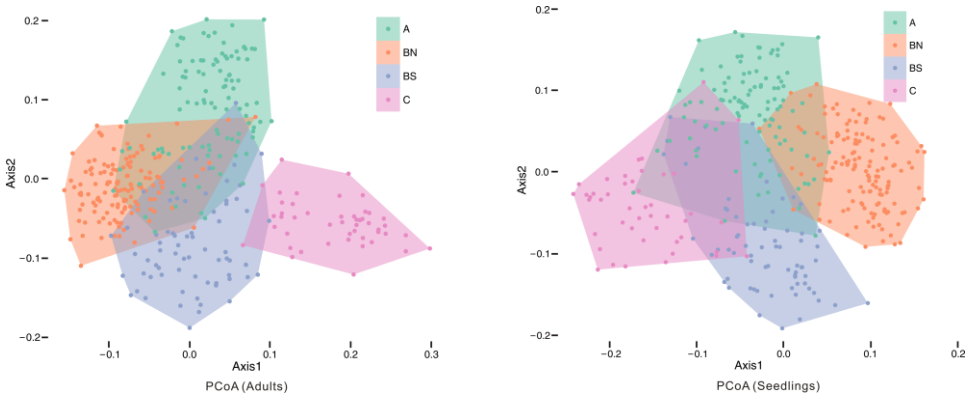

Fig. 4 Principal coordinate analysis (PCoA) of AFLP among the 384 adults (Left) and 384 seedlings (Right) of *Polylepis tarapacana*. The samples are color coded according to their geographic origins. Abbreviations: A, Argentina; BN, Bolivia North; BS, Bolivia South; C, Chile.

TABLE 4 Mantel Tests for the individuals across all four regions for *Polylepis tarapacana*, each of the four regions, and different combinations of each region.

	r_M (Adults, Seedlings)	r_M (Adults)	r_M (Seedlings)
All	0.359**	0.342**	0.364**
BN	0.024	-0.138	0.113
BS	0.2	0.175	0.194
A	0.797*	0.807*	0.621^{+}
C	-0.423	0.53	0.514
BN,BS,C	0.484*	0.835*	0.500*
BN,BS	0.843*	0.576*	0.760*
BS,C	0.544*	0.614*	0.479*
BN,C	0.602*	0.835*	0.585*

Abbreviations: BN, Bolivia North; BS, Bolivia South; A, Argentina; C, Chile; $^{+}$, $0.05 \leq P \leq 0.1$; *, $0.01 < P \leq 0.05$; and ** $P \leq 0.01$.

DISCUSSION

We discovered genetic patterns of one of the world's highest treeline species, *P. tarapacana*, using AFLP markers. First, we found moderate to high genetic diversity and low genetic differentiation, and no significant difference of genetic diversity between life stages (adults and seedlings). Second, we detected four geographical clusters for adults and seedlings with low genetic differentiation. The results indicate that the current genetic pattern of this species is more likely to be caused by Pleistocene climatic oscillations rather than recent human disturbance.

***Effects of human impact*—**Levels of genetic diversity for adults of *P. tarapacana* in the present study did not differ much from those found for *P. australis* adults in Argentina (Hensen et al., 2011), and they were higher than those found for *P. incana* in Ecuador (Hensen et al., 2012). In contrast to the results for *P. incana* in the latter study (Hensen et al., 2012), but in accordance with the results for *P. pauta* in Ecuador (Aragundi et al., 2011), we found similar levels of genetic diversity for *P. tarapacana* adults and seedlings. The result suggests that the genetic diversity of *P. tarapacana* has not been significantly affected by forest fragmentation during the last few hundred years. Nevertheless, as these genetic patterns are certainly also influenced by life history and ecological traits including population sizes, fecundity and longevity, we believe that they mainly reflect historical and geographical signatures in the intensity of human impact on these populations. The distribution of *P. tarapacana* covers the driest and least densely populated region of the (sub)tropical Andes.

This region has been inhabited by humans for millennia (Kessler, 2002), but their effect on the distribution of *Polylepis* forests does not seem to have influenced their genetic diversity. Perhaps most importantly, vegetation cover here is so sparse that fires, the main reason for the large-scale destruction of these forests (Kessler, 1995, 2002), cannot spread. Also, grazing is largely done by native camelids and is concentrated in valley bottoms, such that human influence on *P. tarapacana* takes place mainly via timber extraction. In contrast, in the more humid Argentinean range of *P. australis*, human impact has greatly increased within the last few hundred years (Renison et al., 2006). Here, both the frequent use of fires and grazing with non-native animals (cattle, sheep) within forest patches have had negative impacts on the *Polylepis* forests (Renison et al., 2015). In the Ecuadorian range of *P. incana*, human impact also has a long history and much of the fragmentation probably pre-dates Spanish colonization (Bush et al., 2015), current impacts are also rather pronounced (Cierjacks et al., 2008). Accordingly, the populations of *P. incana* studied in the country showed strong age-related signatures of human impact (Hensen et al., 2012).

We therefore propose that the history of human land use, modulated by different environmental conditions, shows a geographic signature in the age-related genetic diversity of *Polylepis* forests. While more studies are needed to verify our assumptions, they potentially provide a guideline on predicting where human impact may be most detrimental and where corresponding management activities are thus most urgently needed.

***Phylogeographic history*—**Greater genetic variability of genetic diversity was found within, rather than among populations, as has both been found for other *Polylepis* species (Hensen et al., 2011, 2012; Gareca et al., 2013). Our overall fixation indices (F_{ST} values) provide evidence of weak spatial isolation, particularly when regarding the large scale of our study, and are much lower than those determined for *P. australis* (F_{ST} = 0.165 for adults, Hensen et al., 2011) and *P. incana* (F_{ST} = 0.307 for adults, and F_{ST} = 0.298 for seedlings, Hensen et al., 2012). In our study, the low genetic differentiation may be due to the maintenance of still large population sizes (field observation). Gene flow through seeds or pollen between populations might replenish alleles that have been lost through drift, which in turn reduces genetic differentiation between populations (Hensen et al., 2011). In addition, the effect of drift on population structure in tetraploids is probably reduced compared to diploids due to a twice as high effective population size for *P. tarapacana* (Meirmans and Van Tienderen, 2013). It should be noted that the results in our study might be biased due to the unavoidable genotyping error as we used the dominant data. However, we attempted to limit the biases by:

1) reducing the genotyping error as much possible following the suggestions of Pompanon et al. (2005), and 2) comparing genetic diversity and differentiation with those of other *Polylepis* studies with the same AFLP markers.

Despite the overall low level of genetic differentiation, we found four distinct clusters for adults and seedlings (Figs 3 and 4). These four clusters were concordant with the four predefined and non-overlapping geographic regions. We assume that particular Andean landscape structures hampering gene flow, such as high-mountain crests or lower elevation depressions, might explain the clusters. The role of such barriers for gene flow is impressively demonstrated by the highest pairwise F_{ST} values found between the Chilean forests and those of nearby northern Bolivia. While the four Chilean populations are located on the western side of the volcanic chain marking the borderline between Chile and Bolivia (Fig. 1), those of Bolivia and Argentina are located on the eastern side of the chain.

The low genetic differentiation between the four clusters may also be caused by the fact that the populations of *P. tarapacana* expanded to lower elevations and experienced gene flow in continuous populations during the cold glacial periods, with the Chilean populations shifting toward the Pacific and the Bolivian and Argentinean populations migrating toward lower elevations within the Altiplano. During warm interglacial periods the populations may have persisted only in isolated populations in the highlands. In accordance, the fossil record of *Polylepis*-type pollen covering multiple glacial/interglacial periods in the central Andes confirms several vertical migration cycles of *Polylepis* species in response to past climate change events, and a wider distribution in glacial periods (Gosling et al., 2009).

Focusing on within-cluster differentiation, we only detected significant isolation by distance for the Argentinean cluster. This means that the Argentinean forest plots were somewhat more genetically differentiated than those of Bolivia and Chile, suggesting slightly stronger spatial isolation and enhanced genetic drift. In northern Argentina, topography is more divided, and higher mountain crests may represent barriers to pollen and seed flow (Hensen et al., 2011). The absence of isolation by distance across Bolivia and Chile could be accounted for by the relatively weaker landscape boundaries and the more continuous habitat of *P. tarapacana*, and thus pollen could disperse over long distances as found for *P. australis* (Seltmann et al., 2009).

We did not find a correlation between genetic diversity and elevation, which contrasts with previous results for *P. australis* and *P. incana* (Hensen et al., 2011, 2012). We assume that this contrasting pattern is the result of the more continuous elevational distribution of *P. tarapacana*, compared to *P. incana* and *P. australis* where fragments were sampled. Thus,

although *P. tarapacana* most probably migrated up- and downslope during the Pleistocene interglacial-glacial periods, such patterns are not evident in its genetic signature, due to the extensive gene flow in continuous populations along elevations.

In accordance with our initial hypothesis, genetic diversity of *P. tarapacana* decreased toward the equator. In part, this may be related to decreasing aridity, as we also found a negative relationship between annual mean precipitation and genetic diversity in *P. tarapacana*, which explains variation in genetic diversity better than latitude. The pattern of decreasing genetic diversity toward the north is consistent with our previous study of *P. australis*, which has both higher genetic diversity (Hensen et al., 2011) and higher variability of ploidy levels (Kessler et al., 2014) in the southern extent of its range. The genetic pattern of *Podocarpus parlatorei* also revealed the long-term persistence of cold-tolerant elements in the northern Argentinean Andes adjacent to our Argentinean cluster (Quiroga and Premoli, 2007). These findings suggest that southern populations of these species persisted during glacial periods while northern populations would have been relatively young. A possible interpretation is that *P. tarapacana* and other cold-adapted high Andean plant populations may have persisted in northern Argentina because here, the high western Andean range is closer to the eastern cloud forests and is separated by a lower eastern range in comparison to the Bolivian and Chilean populations. In addition, the northern Andes of Argentina are known to harbor higher levels of bird endemism than adjacent regions of Bolivia, which has been linked to historical eco-climatic stability (Sandel et al., 2011). It thus seems likely that this geographical region would have served as a refugium for montane taxa during past climatic shifts.

In conclusion, the geographical patterns of genetic diversity, in particular the high levels of genetic connectivity found in our study, support the idea of a historically more widespread distribution of the treeline species *P. tarapacana*, possibly during cooler Pleistocene periods. In addition, we suggest that the four geographical clusters have and could serve as putative refugium areas or reservoirs of genetic diversity, particularly for Argentinean populations of *P. tarapacana* under a global climate-change scenario.

ACKNOWLEDGEMENTS

Many thanks to Duilio Shinner and Julio Dominguez for all their help during the collection of the samples, to Erik Welk for help producing the map, and Birgit Müller for help in the laboratory. We thank the personnel of CONAF in Chile, especially Hector Ricardo Peñaranda Antezana, for their hospitality and logistical support. We thank the Handling Editor and two

anonymous reviewers for their constructive and thoughtful comments. Yanling Peng was funded by the China Scholarship Council (CSC, No. 2011618106); and Daniel Renison was funded by CONICET Argentina.

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Chapter 5

Range expansion during the Pleistocene drove morphological radiation of the fir genus (*Abies*, Pinaceae) in the Qinghai-Tibet Plateau and Himalayas

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Botanical Journal of the Linnean Society (2015). 179(3): 444-453

ABSTRACT

Range expansion caused by climate oscillations in the past probably promoted morphological radiation in a few plant groups. In this study, we aim to test this hypothesis through phylogeographic analysis of the cold-tolerant fir genus (*Abies*) in the Qinghai-Tibet Plateau (QTP) and Himalayas, where it comprises 12 described species. We examined sequence variations in two maternally inherited mitochondrial (mt) DNA fragments (*nad5-4* and *nad7-1*) and two paternally inherited chloroplast (cp) DNA fragments (*trnS-G* and *trnL-F*) for 733 individuals from 75 populations of the species within a monophyletic clade. Only six mtDNA haplotypes were recovered, but five of them were shared between multiple species and one occurred at a high frequency, providing strong evidence of range expansion. A total of 43 cpDNA haplotypes were detected, of which 19 were shared between species and three occurred at high frequency. Network, mismatch, and Bayesian skyline plot analyses of all cpDNA haplotypes from this clade clearly suggested range expansion. This expansion was dated as having occurred during the longest and most extensive glaciation in the Pleistocene. Our results therefore supported the range expansion hypothesis for this fir clade during the Pleistocene; expansion probably drove the morphological radiation of the clade in the QTP and Himalayas although it remains unclear whether the different morphotypes should be acknowledged as independent, reproductively isolated species.

Keywords: *Abies*, climate oscillations, expansion, phylogeography, Qinghai-Tibet Plateau and Himalayas

INTRODUCTION

Range expansion after colonization of new areas, especially islands, has resulted in extensive morphological radiation, with numerous species of both animals and plants described as having undergone this process (Darwin, 1859; Carlquist, 1970). Climatic oscillations during the Pleistocene have been found to have caused range expansions and retreats of most species that occurred in climate-sensitive regions (Avise, 2000; Hewitt, 2004). In both North America and Europe, refugial isolation during these climate changes was found to have caused morphological divergence and recent speciation in some groups (Schluter, 2000; Coyne & Orr, 2004; Weir & Schluter, 2004). However, we still have a poor understanding of what roles range expansion played in driving morphological radiation leading to numerous morphotypes (but see Milá *et al.*, 2007). Range expansion caused by climatic oscillations (Avise, 2000) probably promoted morphological radiation, producing numerous morphotypes or species within one clade (Milá *et al.*, 2007). Under this scenario of range expansion-morphological radiation, ancestral genetic polymorphisms dominate across multiple current species or morphotypes because of incomplete lineage sorting (Milá *et al.*, 2007). These shared polymorphisms differ from those caused by gene flow (e.g. Whittemore & Schaal, 1991; Dumolin-Lapegue, Kremer & Petit, 1999) in that they are usually ancestral to the morphotype-specific alleles (Milá *et al.*, 2007).

Here we test this hypothesis of range expansion-morphological radiation triggered by climate oscillations in the past through phylogeographic analysis of one group of closely related fir species occurring in the Qinghai-Tibet Plateau (QTP) and the Himalayas. These two regions, which comprise the "world's third pole", are believed to be highly sensitive to past and future climatic changes (Zheng, 1996). In addition, the extensive uplifts that the QTP has experienced since the early Miocene are likely to have brought about a series of climate alterations (Shi, Li & Li, 1998). Both geological and climatic changes have contributed to an increase in the diversity of plants in this high-altitude region (Wu *et al.*, 1988), which is known to contain the most diverse alpine flora in the world (Wu & Wu, 1996). The eastern part of the QTP and the adjacent regions are listed as one of the world's biodiversity hotspots because of the high level of species richness (Myers *et al.*, 2000). Most alpine genera, and those preferring low temperatures, have undergone extensive diversification in this region (Wang *et al.*, 1993; Wu & Wu, 1996; Liu *et al.*, 2006; Xu *et al.*, 2010; Sun *et al.*, 2012). In addition, phylogeographic analyses of some species at the population level have suggested that alpine species were particularly sensitive to climate oscillations during the Pleistocene;

the results showed clear patterns indicative of range retreat and expansion (e.g. Zhang *et al.*, 2005; Wang *et al.*, 2009). It is therefore highly likely that range expansions triggered by the Pleistocene climate change may also have promoted morphological (or species) radiation in some groups.

The fir (*Abies*) genus consists of *ca.* 50 species which are mainly distributed in the cold temperate and boreal regions of the Northern Hemisphere (Farjon, 1990). This genus seems to have undergone only recent diversification in species morphology, given the lack of complete reproductive isolation (Linares, 2011) and the long generation time (around 25 years) (Farjon, 1990). Twenty-two species are found in China, of which about 70% occur in, and 50% are endemic to, the QTP and Himalayas (Fu, Li & Elias, 1999; Xiang, Cao & Zhou, 2007). Fir pollen fossils in the QTP can be dated back to the Miocene, but they became more abundant in the Quaternary (Li & Wu, 1978; Wang, 1992; Xiang, Cao & Zhou, 2007). The species occurring in these regions have distinctly diverse morphological traits, and they have been tentatively grouped into either three or two different sections (Liu, 1971; Farjon & Rushforth, 1989). However, this classification has received little support from phylogenetic analysis based on ITS sequence variation because the species sampled did not group into subclades despite comprising a tentative clade (Xiang *et al.*, 2009). Further phylogenetic analyses based on sequence variations in multiple chloroplast DNA fragments suggested that all species sampled from the QTP and Himalayas comprised a clade but that there was no distinct genetic differentiation between them (Semerikova & Semerikov, 2014; Xiang *et al.*, 2015). This clade probably diverged from its sister clade in the late Miocene (Xiang *et al.*, 2015), although the process of species diversification within the clade remains unknown. The lack of genetic differentiation between species (Semerikova & Semerikov, 2014; Shao & Xiang, 2015; Xiang *et al.*, 2015) led us hypothesize that these high-altitude fir 'species' might have arisen from recent morphological diversification following a common range expansion, probably triggered by the glacial climate during the Pleistocene, when the cold-tolerant firs became widely distributed in these regions. In order to test this expansion-diversification hypothesis, in the present study we conducted a phylogeographic study of 12 closely related fir species in the QTP and Himalayas. We used mitochondrial (mt) and chloroplast (cp) DNA sequence variation to examine phylogeographic patterns in this fir clade. As in other conifers, in fir species mtDNA is maternally inherited and dispersed through seeds, while cpDNA is paternally inherited and transmitted by pollen and seeds (Neale & Sederoff, 1989; Petit *et al.*, 2005). Sequence variations in these organelle genomes have been widely used to examine

both range changes within individual species and interspecific divergence between species that co-occur within local regions (e.g. Tsumura & Suyama, 1998; Liepelt, Bialozyt & Ziegenhagen, 2002; Ziegenhagen *et al.*, 2005; Jaramillo-Correa *et al.*, 2008; Liepelt *et al.*, 2010; Jiang *et al.*, 2011; Wang *et al.*, 2011; Aguirre-Planter *et al.*, 2012; Peng *et al.*, 2012; Shao & Xiang, 2015; Xiang *et al.*, 2015). Specifically, our research aimed to address the following two questions. (1) Do sequence variations in the DNA fragments sampled for this fir clade, which occurs in the QTP and Himalayas and displays extensive morphological diversification, show a clear pattern of demographic expansion? (2) If so, could this expansion be dated to within the Pleistocene, when the region experienced extensive climate change (Shi, Li & Li, 1998; Yi, Cui & Xiong, 2005)?

MATERIAL AND METHODS

SPECIES DESCRIPTION AND SAMPLING

Over the past 10 years, we have collected 733 individuals from 75 populations representing 12 fir species occurring in the QTP and Himalayas. All these species occupy different niches (Supplementary Table S1). Of these samples, more than 5 populations and more than 45 individuals of each of these populations were sampled for each of 8 species, i.e. *A. spectabilis* Spach, *A. recurvata* Masters, *A. squamata* Masters, *A. georgei* Orr, *A. forrestii* Coltman Rogers, *A. fabri* Craib, *A. nukiangensis* W.C. Cheng & L.K. Fu, and *A. ernestii* Rehder; for the remaining 4 species, i.e. *A. densa* Griffith, *A. pindrow* Spach, *A. delavayi* Franchet, and *A. ferreana* Bordères & Gaussen, which have narrow distributional ranges, 2 to 4 populations and fewer than 20 individuals of each population were sampled. Sample sizes differed among species, depending on their distributional ranges and individual abundance in the field. The distance between any two individuals sampled within each population was at least 100 m. Fresh needles were put in paper bags and dried immediately in the field with silica gel. Voucher specimens of all populations of the 12 fir species were stored at Lanzhou University (herbarium code: LZU). A hand-held eTrex GIS (Garmin) was used to measure the latitude, longitude, and altitude of each sampling site. Sampling details are provided in Fig. 1A and Supplementary Table S1.

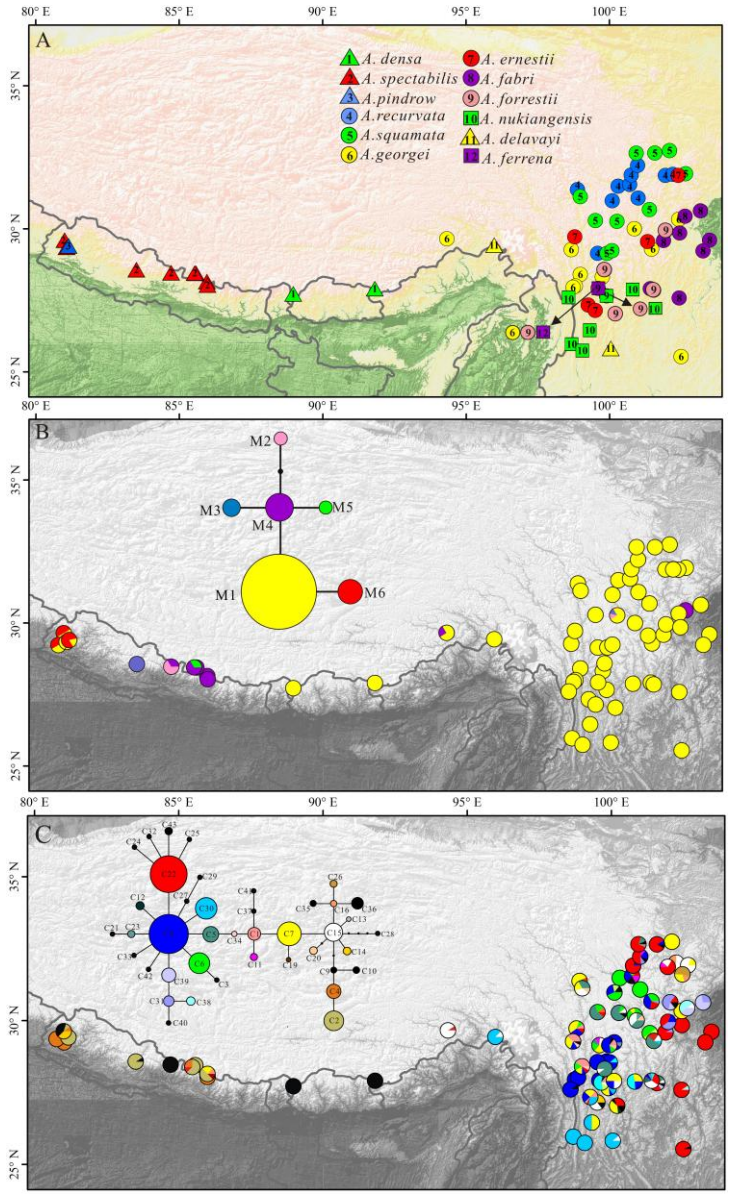

Fig. 1 (A) Distribution of the sampled populations of 12 taxa of the genus *Abies* across the QTP and Himalayas; (B) distributions and networks of six mitotypes; (C) distributions and networks of 43 chlorotypes. The area of each circle in the networks is proportional to the frequency of occurrence.

SEQUENCES AND PHYLOGEOGRAPHIC ANALYSES

Two mtDNA loci, *nad5-4* and *nad7-1*, and two cpDNA loci, *trnS-G* and *trnL-F*, were sequenced for all individuals following the methods we described previously (Peng *et al.*, 2012). Sequence variations in these fragments have been used to distinguish different fir species and examine interspecific variation (e.g. Jiang *et al.*, 2011; Wang *et al.*, 2011; Peng *et al.*, 2012). Multiple alignments of sequences were produced using MEGA5 (Tamura *et al.*, 2011) and checked manually. All newly determined sequences have been deposited in the

NCBI GenBank database under Accession Numbers (*nad5-4*: KR606688 - KR606708; *nad7-1*: KR606709 - KR606721; *trnL-F*: KR606722 - KR606759; *trnS-G*: KR606760 - KR606818). The two linked mtDNA loci were concatenated, as were the two linked cpDNA loci, and each pair was treated as one sequence in all analyses. All indels were coded as binary characters (0 or 1) using the GapCoder program (Young & Healy, 2003). To evaluate genealogical relationships among sequences with shallow levels of genetic divergence, an unrooted network based on statistical parsimony was created using TCS version 1.21 (Clement, Posada & Crandall, 2000) according to the acceptance criteria outlined in Templeton, Crandall & Sing (1992). A default parsimony connection limit of 95% was used.

Molecular diversity indices, including the number of haplotypes (*nh*), haplotype diversity (*H*d), and nucleotide diversity (*Nd*), were estimated for each species and each population using Arlequin 3.5 (Excoffier & Lischer, 2010). Hierarchical AMOVA was performed to calculate the variance partitioning at three levels among species, among populations within species, and within populations. For comparison of genetic diversity between different species, genetic differentiation (F_{ST}) was also assessed for all pairs of species following AMOVA. The significances of all these analyses were determined in the software package Arlequin 3.5 (Excoffier & Lischer, 2010), using a permutation procedure with 1000 runs.

TESTS FOR DEMOGRAPHIC EXPANSION

We tested demographic expansion on the basis of cpDNA data alone, due to the limited number of variations in the mtDNA, using the following methods. Firstly, Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) tests for departure from neutrality were calculated using Arlequin 3.5 (Excoffier & Lischer, 2010) with 1000 bootstrap replicates. Under the expansion hypothesis, the *Fs* statistic would have a large negative value within a given area due to an excess of rare new mutations, while Tajima's *D* value would be negative under conditions of population expansion (Aris-Brosou & Excoffier, 1996). Secondly, mismatch distribution analysis (MDA) based on the number of segregating sites, under the sudden demographic and the spatial expansion model, were determined in Arlequin 3.5 (Excoffier & Lischer, 2010) with 1000 bootstrap replicates. We estimated the time of expansion from the distribution of pairwise nucleotide differences among individuals based on cpDNA haplotypes with the equation $\tau = 2ut$ (Rogers & Harpending, 1992), where t is the expansion time in number of generations, τ is the mode of the mismatch distribution, and u is the mutation rate for the whole haplotype. The u value was calculated according to $u = \mu kg$; in this equation, μ is the

silent divergence·site^{-1}·year^{-1} (s·s^{-1}·y^{-1}), *k* is the average sequence length of the DNA region, and *g* is the generation time in years. We assumed a generation time of 25 years (Farjon *et al.*, 1990). The cpDNA mutation rate in fir species was assumed to be μ = 2.61-4.02 × 10^{-10} s·s^{-1}·y^{-1} (Gernandt *et al.*, 2008). However, neutrality tests and MDAs of DNA variations are sometimes unable to take all demographic signals into account because they rely solely on segregating sites and haplotype patterns (Fitzpatrick *et al.*, 2009). Demographic dynamics were therefore further inferred from Bayesian skyline plot (BSP) analyses carried out using BEAST V. 1.7.5 (Drummond *et al.*, 2005). Piecewise-constant models were explored using an uncorrelated lognormal relaxed clock. Runs consisted of 20,000,000 generations, with trees sampled every 1000 generations. The BSP was visualized in the program Tracer, which summarizes the posterior distribution of population size over time.

RESULTS

MTDNA VARIATION AND MITOTYPE DISTRIBUTION

Two variants resulted from one indel in the *nad*7-1 region, while five variants were produced by one indel and four substitutions in *nad*5-4. The alignment of concatenated sequences was 959 bp in length, giving a total of six mitotypes (M1-M6). The relationships among mitotypes are shown in the minimum spanning network produced by TCS 1.21 (Fig. 1B). Two mitotypes, M1 (frequency > 89%, Supplementary Table S2) and M4 (frequency > 6%), occupied the central positions in the network, while the remaining four, rare, mitotypes M2, M3, M5 and M6 (frequency < 2% each) were located at the tips (Fig. 1B, Supplementary Table S2).

Among the six mtDNA haplotypes obtained, five were shared between two or more species, while one was found only in *A. spectabilis* (Supplementary Table S2). One of the five shared mitotypes, M1, was present in all the species surveyed, and was fixed in most populations with the exception of one of the *A. fabri* populations and six of the *A. spectabilis* populations. One mitotype, M4, was shared by three species, being present in four *A. spectabilis* populations, one *A. georgei* population and one *A. fabri* population. Three mitotypes were shared by two species: mitotypes M2 and M3 both occurred in one *A. spectabilis* population and one *A. georgei* population, while mitotype M6 occurred in one *A. spectabilis* population and one *A. pindrow* population.

Table 1 AMOVA of mtDNA and cpDNA variations in *Abies* taxa across the QTP and Himalayas.

Source of variation	SS	VC	PV (%)	Fixation indices
mtDNA				
Among the 12 taxa	28.991	0.03356 Va	27.52	F_{CT} = 0.276***
Among populations within taxa	41.515	0.06508 Vb	53.64	*F*sc = 0.741***
Within populations	15.057	0.02274 Vc	18.75	F_{ST} = 0.812***
cpDNA				
Among the 12 taxa	310.372	0.41702 Va	36.74	F_{CT} = 0.367***
Among populations within taxa	239.663	0.35416 Vb	31.2	*F*sc = 0.493***
Within populations	239.412	0.36385 Vc	32.06	F_{ST} = 0.812***

Abbreviations: SS, sum of squares; VC, variance component. ***P* < 0.001, 1000 permutations.

Seven of the 75 populations surveyed were polymorphic, whereas the remaining populations contained only one mitotype (Fig. 1B, Supplementary Table S1). No intraspecific mtDNA variations were recorded in eight species, *A. densa, A. recurvata, A. squamata, A. ernestii, A. forrestii, A. nukiangensis, A. delavayi,* and *A. ferreana*, each of which had one mitotype fixed. Intra-specific polymorphisms were found in the remaining four species, *A. spectabilis, A. pindrow*, *A. georgei* and *A. fabri* (Supplementary Table S2).

AMOVA showed that the differences among species explained about 28% (F_{CT} = 0.276), those among populations within species about 54% (F_{SC} = 0.741), and those within populations about 19% of the total mtDNA variation (F_{ST} = 0.812) (Table 1). In most cases pairwise F_{ST} values among species were low (Supplementary Table S3).

cpDNA VARIATION AND CHLOROTYPE DISTRIBUTION

The *trn*S-G alignment was 744 bp long and the *trn*L-F alignment was 423 bp long, with 20 and 11 variants respectively. The alignment of the concatenated sequence produced by combining the two fragments was 1145 bp in length, giving a total of 43 chlorotypes (C1-C43). Of these, 24 were species-specific and thus 19 were shared between species (Supplementary Table S2). Most chlorotypes were arranged as a radiative phylogeny relative to the central ones (for example, C7, C8, C15 and C22) which occurred at high frequency. Almost all rare haplotypes at the tips of the network were species-specific (Fig. 1B).

Twenty-two populations were fixed for a single chlorotype, while the remaining 53 were polymorphic (Fig. 1C, Supplementary Table S1). Seven chlorotypes, C2, C6, C7, C8, C15,

C22, and C30, were common (frequency > 4%); the remaining 36 chlorotypes were rare (frequency < 4%; Supplementary Table S2). Low or no intra-specific variation was detected in three species: *A. densa* and *A. ferreana*, each of which had only one chlorotype, and *A. delavayi* (*H*e, 0.22). In contrast, higher genetic diversity was detected for the other nine species (0.56 < *H*e < 0.84) (Supplementary Table S2).

AMOVA showed that the differences among species explained about 37% (F_{CT} = 0.367), those among populations within species about 31% (F_{SC} = 0.493), and those within populations about 32% (F_{ST} = 0.679) of the total cpDNA variation, respectively (Table 1). In most cases pairwise F_{ST} values among species for cpDNA variation were low, but higher than those for mtDNA variation (Supplementary Table S3).

Table 2 Mismatch analyses and neutrality statistics for cpDNA in *Abies* taxa from the QTP and Himalayan regions combined.

Demographic expansion			Spatial expansion			Neutrality test	
T	*SSD* (*P*)	*HRag* (*P*)	T	*SSD* (*P*)	*HRag* (*P*)	Fu's *Fs* (*P*)	Tajima's *D* (*P*)
3.828	0.0045 (0.18)	0.0348 (0.125)	3.832	0.0045 (0.142)	0.0348 (0.135)	-25.93 (0)	-1.44 (0.037)

Abbreviations: *SSD*, sum of square deviations; *HRag*, raggedness index.

DEMOGRAPHIC EXPANSION

A significantly negative value of Fu's *Fs* statistic was found for fir species in the QTP and Himalayas groups (-25.93, *P* < 0.001; Table 2). A negative value (-1.44) was also obtained for Tajima's *D* criterion, indicating that there had been a demographic expansion. The observed mismatch distributions based on both expansion models fitted all the individuals from the QTP and Himalayas, a result indicative of demographic or spatial expansion (Fig. 2A,B, Table 2). Based on the substitution rate, generation time, and different expansion models assumed for the fir genus, it was estimated that the expansions occurred between 190 and 293 thousand years ago (Kya) for both the QTP and the Himalayas. Our BSP analysis also indicated range expansion in the past (Fig. 2C). In this case the expansions were estimated to have taken place 900 Kya for the QTP and Himalayas, based on units of substitution per site (shown on the x-axes of the BSP analysis) and the average mutation rate (Fig. 2C).

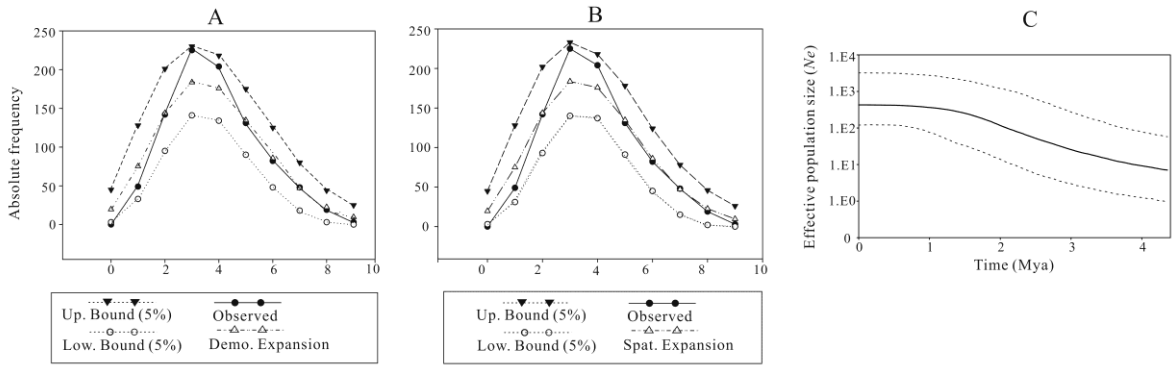

Fig. 2 Mismatch distributions under a demographic expansion model (A) and a spatial expansion model (B) and Bayesian skyline plots (C) for all species examined from 12 taxa of one *Abies* clade from the QTP and Himalayas.

DISCUSSION

The species sampled have distinct morphological traits, to such an extent that they have been placed in different sections (Liu, 1971; Farjon & Rushforth, 1989). However, we found that sequence variations in the two mtDNA and two cpDNA fragments used in this study could not distinguish between the 12 species occurring in the QTP and Himalayas, although some rare chlorotypes were species-specific. Little genetic differentiation between species was detected using either mtDNA or cpDNA markers (Table 1, Supplementary Table S3). Most populations of the species sampled are dominated by a single mitotype or by common chlorotypes, while other mitotypes and chlorotypes present at low frequencies are clearly derived from these dominant haplotypes (Fig. 1B,C). It has been assumed that haplotypes that are shared between species and regions derive from the common ancestral species, either due to incomplete lineage sorting or as a result of introgression by gene flow after speciation (e.g. Wu, Krutovskii & Strauss, 1998; Bouillé & Bousquet, 2005; de Queiroz, 2007; Wiens, 2007; Currat *et al.*, 2008; Petit & Excoffier, 2009). Three evolutionary histories could in theory have accounted for the phylogeographic patterns observed here for the 12 species. First, hybridization events due to secondary contact after initial divergence could have caused the sharing of mitotypes and chlorotypes between species observed here. However, haplotypes that are shared due to hybridization are usually distributed in the regions of contact of two species and are present at relatively low frequency (de Queiroz, 2007; Currat *et al.*, 2008; Petit & Excoffier, 2009). We found that most shared haplotypes are frequent in the species

concerned, suggesting that hybridization probably contributed little to the shared polymorphisms. Second, there may have been a sweep across the already differentiated fir species by those haplotypes with highly adaptive values, repeatedly crossing hybrid zones between the 12 species through interspecific gene flow. It is well known that evidence for selective sweeps is manifested only in a few loci that are under selection, or in loci tightly linked to them, whereas bottlenecks and range expansions affect the entire genome equally (Galtier, Depaulis & Barton, 2000). In this study, mitotypes and chlorotypes, which have different modes of inheritance, showed similar biogeographic patterns, and it is unlikely that such an adaptive sweep shaped the patterns observed across these two genomes at the same time. Finally, the shared polymorphisms might have derived from incomplete lineage sorting before species divergence (Avise, 2004). It is likely that all the species sampled experienced a common expansion, which resulted in the widespread fixture of the ancestral polymorphisms, before they underwent morphological diversification. In addition to the widespread fixing of a single haplotype, this range expansion also produced a few derived haplotypes at low frequencies in the region colonized (Echt *et al.*, 1998). This 'star-like' phylogeny of haplotypes derived from putative ancestral ones (Avise, 2004) due to common expansion was observed for both chlorotypes and mitotypes (Fig. 1B,C). The common expansion hypothesis was further supported by the unimodal mismatch distribution (Fig. 2A,B), BSP analyses (Fig. 2C) and the negative Fu's F_S* and Tajima's *D* statistics obtained for cpDNA data (Table 2) (Aris-Brosou & Excoffier, 1996). All available analyses seem to support the hypothesis that a common expansion occurred before morphological diversification of this fir clade in the QTP and Himalayas, although we are uncertain whether these morphotypes should be acknowledged as independent reproductively isolated 'species' (Fu *et al.*, 1999).

The range expansion was dated as having occurred approximately between 200 and 900 Kya, within the Pleistocene. The generation times and mutation rates of the sampled fir species are not known. There is a high level of uncertainty attached to all these time estimates and the fact that they greatly differed from each other means that the dating should be treated with caution. However, the estimates provided by mismatch distributions and BSP fall within the same range and it should be noted that this period was during the most extensive and longest of the glaciations that occurred in the QTP and Himalayas (Shi, Li & Li, 1998; Yi, Cui & Xiong, 2005). In addition, fossil pollen has indicated that cold-tolerant fir species expanded extensively and continuously in high-altitude regions in response to the cold climate during this period (Li & Wu, 1978; Wang, 1992; Xiang, Cao & Zhou, 2007). The dominant

haplotypes were therefore widely distributed across these regions, and haplotypes derived from these ancestral ones were produced during the subsequent evolution and divergence. During the warming that followed the glacial age, the previously continuous fir forest probably became restricted to numerous patches on mountains separated by deep valleys, and this is likely to have led to rapid morphological diversification in the QTP and adjacent regions, as has been found for other plants (Liu *et al.*, 2006; Xu *et al.*, 2010). However, this morphological differentiation took place relatively recently, in the period since the Pleistocene; this, coupled with the long generation time of fir species, means that these morphotypes still retain the dominant haplotypes of their ancestors, together with morphotype-specific endemic haplotypes at low frequencies. A low level, or even absence, of genetic differentiation was also reported for fir species in this clade based on other cpDNA, mtDNA or nuclear DNA fragments (Xiang *et al.*, 2009, 2015; Semerikova & Semerikov, 2014; Shao & Xiang, 2015), similarly suggesting that this clade has undergone recent morphological diversification. However, more pronounced range shrinkages, especially in response to the warm period after the LGM and earlier interglacial stages, may have resulted in the more rapid lineage sorting and interspecific divergence that occurred between the fir congeners in Europe or North America, where examination of sequence variations at these fragments has shown that fewer haplotypes are shared between species than is the case for the species in our study (e.g. Liepelt, Bialozyt & Ziegenhagen, 2002; Jaramillo-Correa *et al.*, 2008; Liepelt *et al.*, 2010).

Overall, our findings suggest that the populations that we sampled across different morphotypes (species) experienced a common expansion triggered by climate change during the Pleistocene. Following this expansion, this fir clade probably underwent rapid morphological diversification in the QTP and Himalayas, to produce the present range of morphotypes, or taxonomic 'species' as they are currently described by Fu *et al.* (1999), although the extent of reproductive isolation between them remain unknown. However, the radiative species diversification of the other species-rich genera in this region seem to have occurred earlier than the date which we have recovered here for the fir species: their diversifications have been dated to the periods when the QTP began its extensive uplifts between 10-15 Mya, 7-8 Mya, and 3-5 Mya (Zhang *et al.*, 2005; Liu *et al.*, 2006; Wang *et al.*, 2009; Sun *et al.*, 2012). It will be interesting to discover when and how other species-rich (or morphotypes-rich) genera which have not yet been examined began their morphological

radiations, and the extent to which these radiations have contributed to the status of the QTP and Himalayas as a biodiversity hotspot.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Key Project for Basic Research (2014CB954100) and the National Natural Science Foundation of China (30725004). We thank the China Scholarship Council (CSC, No.2011618106) for supporting Yanling Peng's stay in Germany. We are grateful to the Sees-Editing company for editing the English of the manuscript.

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Chapter 6

Synthesis

General discussion

Four case studies were undertaken to elucidate the impacts of clonal propagation, elevational gradients, human disturbance, and Pleistocene climatic oscillations on genetic diversity and structure, as well as diversification of several high-elevation tree species from fine to large scales. The main conclusions are summarized in Figure 6.

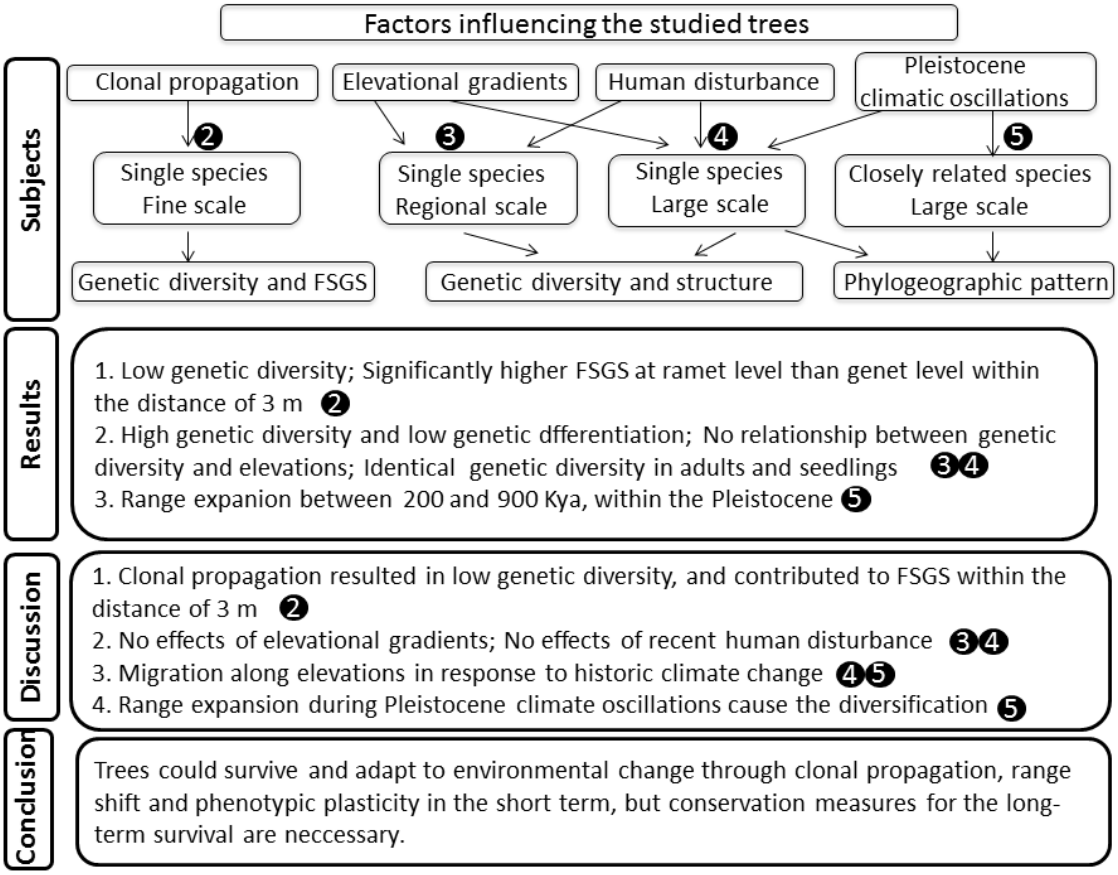

Fig. 6 Overview of the subjects, main results, and main topics of discussion and conclusions of this dissertation. Numbers in circles correspond to chapter numbers.

Clonal propagation

The study presented in Chapter 2 indicates that clonal propagation, combined with the effect of limited gene flow due to reduced sexual reproduction and genetic drift due to small population size, result in a low genetic diversity in *P. reticulata* under harsh environmental conditions (Fig. 6). This result supports the idea that clonal propagation may result in reduced fitness over time due to increased levels of selfing and inbreeding depression in self-compatible species (Honnay & Jacquemyn 2008; Seltmann *et al.* 2009), rather than the idea that clonal propagation may increase the effective number of alleles and heterozygosity in a population (e.g., Balloux *et al.* 2003). However, in the case of vulnerable species under harsh

environmental conditions, clonal propagation could be beneficial for the survival of the species in the short term (Linhart & Gehring 2003; Honnay & Bossuyt 2005; Macek *et al.* 2010; de Witte *et al.* 2012). In the long term, diminished genetic diversity may cause a loss of fitness and evolutionary capacity to adapt to environmental changes.

Effect of elevation gradients

The studies presented in both Chapter 3 and Chapter 4 indicate that there are no effects of elevational gradients on the genetic diversity and structure of *P. australis* and *P. tarapacana* (Table 3). The results highlight the lack of an elevational effect on genetic diversity as a result of an unrestricted gene flow among populations along this environmental gradient (e.g., Truong *et al.* 2007; Table 3). However, a prior study of *P. incana* showed decreasing genetic diversity with increasing elevation (Table 3), which directly supports the hypotheses that this species has experienced genetic bottleneck due to upward range expansion (e.g., Quiroga *et al.* 2002). The extensive gene flow along elevational gradients is helpful for the maintenance of high genetic diversity and low genetic differentiation, which allows trees to adapt to environmental changes.

In addition, the study presented in Chapter 3 did not support the idea that local adaptation occurred along elevational gradients for *P. australis* populations characterized by phenological differences in flowering. This result indicates that the extensive gene flow along elevational gradients could counteract adaptation through the constant influx of maladapted genotypes, a situation under which only strong selection pressure can lead to local adapation (Frei *et al.* 2014). It also supports the conclusion of a recent meta-analysis of other plant species that local adaptation is less common than generally assumed (Leimu & Fischer 2008). The result also lends credence to the idea that through phenotypic plasticity the species is likely to adapt to the different conditions imposed by the elevational gradients. Plasticity may buffer the detrimental effects of rapid climate change – at least in the short term – by allowing time for evolutionary changes (Frei *et al.* 2014). However, it should be noticed that the limited genetic divergence among populations could be caused by the neutral markers AFLP. Further studies on this species should therefore focus on the genetic basis of adaptation using more informative markers such as Resitriction Site Associated DNA markers (RAD), and reciprocal transplant experiments.

Human disturbance

The studies presented in Chapters 3 and 4 indicate that fragmentation has not affected the patterns of genetic diversity of *P. australis* and *P. tarapacana* (Chapter 3-4). The results confirm that tree populations may be resilient to the effects of habitat fragmentation because of the long generation time and extensive pollen dispersal of wind-pollinated trees even in fragmented habitats (Hamrick 2004; Kramer *et al.* 2008). However, negative effects on seed quality resulting from such fragmentation were found in *Polylepis* species (e.g., Seltmann *et al.* 2009). Previous studies of *P. besseri*, *P. incana*, *P. multijuga* and some populations of *P. australis* indicated that fragmentation resulted in low genetic diversity and strong genetic structure in these species (Table 3). All together, the different genetic consequences of fragmentation on the different *Polylepis* species is most likely to be caused by varying extents of fragmentation, which indicate that these tree species could adapt to moderate environmental changes but not extreme ones.

Table 3. Overview of recent studies that addressed the effects of elevational gradients, human disturbance and historic climate changes on patterns of genetic diversity in *Polylepis* species.

Species	Elevational gradients	Human disturbance	Historic climate change	References
P. australis	No	No		Chapter 3
P. tarapacana	No	No	Yes	Chapter 4
P. besseri		Yes		Gareca *et al.* (2013)
P. incana	Yes	Yes	Yes	Hensen *et al.* (2012)
P. multijuga		Yes	Yes	Quinteros-Casaverde *et al.* (2012)
P. australis	No		Yes	Hensen *et al.* (2011)
P. australis		No		Julio *et al.* (2011)
P. australis		Yes		Julio *et al.* (2008)

Yes: factor had an effect; No: factor had no effect; blank: not tested.

Pleistocene climatic oscillations

The studies presented in Chapter 4 indicate that *P. tarapacana* experienced gene flow between populations that resulted in low genetic differentiation during glacial periods. The findings support the hypothesis that species at high elevations expanded to lower elevations and experienced gene flow in continuous populations during the cold glacial periods (Stewart *et al.* 2010). This hypothesis was supported by Chapter 5 and previous studies of other *Polylepis* (Table 3) and *Abies* species (Jaramillo-Correa *et al.* 2008). However, under future global climate warming caused by human activities, the populations of *Polylepis* species and

Abies would be isolated at higher elevations, resulting in reduced gene flow among populations and thus low genetic diversity and high genetic differentiation, which may reduce the ability of populations to adapt.

In addition, the phylogeographic patterns of the genus *Abies* in the QTP and Himalayas (Chapter 5) support the hypothesis that range expansion caused by climatic oscillations in the past probably promoted morphological radiation in some taxonomic groups (e.g., Milá *et al.* 2007), rather than the hypothesis that refugial isolation during the Pleistocene climatic oscillations caused morphological radiation (Schluter 2000; Coyne & Orr 2004; Weir & Schluter 2004). However, the radiative species diversification of the other species-rich genera in this region seems to have occurred earlier than the date which we have recovered here for the fir species: their diversifications have been dated to the periods when the QTP began its extensive uplifts between 10-15 Mya, 7-8 Mya, and 3-5 Mya (Zhang *et al.* 2005; Liu *et al.* 2006; Wang *et al.* 2009; Sun *et al.* 2012). The genus *Abies* in these regions provides an excellent model to test the hypothesis of 'range expansion – morphological radiation' and provide empirical case for understanding evolutionary processes underlying the diversification of species.

Conclusions

This dissertation revealed that four different factors influenced the genetic diversity and structure, as well as the diversification of *Polylepis* and *Abies* species based on four cases that covered multiple spatial scales. In conclusion, the vulnerable species *P. reticulata* revealed a low level of genetic diversity, while *P. australis* and *P. tarapacana* showed high genetic diversity and weak genetic structure. Clonal propagation probably reduces genetic diversity, but is beneficial for the survival of *P. reticulata* under harsh environmental conditions in the short term. Elevational gradients have not affected the genetic diversity and structure for *P. australis* and *P. tarapacana* due to the extensive gene flow along elevational gradients. Moreover, phenotypic plasticity of *P. australis* along elevation gradients could be beneficial for responding to future environmental changes. However, further studies should be conducted on the genetic basis of adaptation for *P. australis*. On the one hand, there is no negative effects of fragmentation on the genetic diversity of the studied *P. australis* and *P. tarapacana* poulations; on the other hand, it seems genetic connectivity during glacial periods has promoted genetic diversity of *Polylepis* and *Abies* species, and range expansion during glacial periods has caused the diversification of *Abies* in the QTP and Himalayas. In the face of global climate warming, populations of *Polylepis* and *Abies* species are expected be

isolated at higher elevations, resulting in low genetic diversity and high genetic differentiation among populations. Thus, to better adapt to furture environmental changes, it is essential to provide conservation strategies for these studied species in order to maintain high genetic diversity or at least maintain the current level of genetic diversty.

The dissertation helps design species conservation and management programs, such as *in situ* and *ex situ* conservation or seed collection for germplasm conservation. For vulnerable *Polylepis* species (e.g., *P. reticulata*), understanding the effects of clonal propagation on FSGS could optimize future sampling designs and avoid the collection of clone mates for *ex situ* conservation (Chapter 2). For *Polylepis* species with large population sizes, for example, the design of conservation programs should consider the definition of genetic management units: the potential for pollen dispersion over large distances found in *P. australis* suggests the necessity of defining management units at landscape level rather than at the level of locally restricted and fragmented populations (Chapter 3); whereas for *P. tarapacana*, four geographical clusters have served and could be maintained as putative refugia or reservoirs of genetic diversity, which could be regarded as four genetic units for conservation (Chapter 4).

The dissertation also provides an empirical basis for understanding evolutionary processes underlying the diversification of the *Abies* species in the QTP and Himalayas (Chapter 5). However, the evolutionary mechanisms that have driven diversification of the genus *Polylepis* in the Andes are still poorly understood. It will be interesting to understand how the species of the genus *Polylepis* started their morphological radiation and the extent to which such radiation of the genus *Polylepis* has contributed to the status of the Andes as a biodiversity hotspot.

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Acknowledgements

Acknowledgements

There are many people who have accompanied me during my doctoral work. Without their support, I would not have finished my doctoral work successfully. Many thanks to all of them.

First, I would like to thank my supervisor Prof. Isabell Hensen for discussing the manuscript and even for modifying my presentation for conferences, and giving me chance to gain a broader view of Andean vegetation. It was she who told me that I should control the balance the work and life. I am really appreciative of her help in the last four years. I am also very grateful for the help of Prof. Jianquan Liu, as he recommended me to Prof. Isabell Hensen and also gave his constructive suggestions on the manuscript.

I owe gratitude to Frau Birgit Mueller and Frau Christine Voigt for their support during the laboratory work. I would like to expand my thanks to all my friends and colleagues from Botanical Garden, Amira Apaza Quevedo, Anne Piel, Baoulh Al-Hawija, Christoph Rosche, Dennis Lippok, Erik Welk, Gunnar Seidler, Heidi Hirsch, Karin Schrieber, Monika Patzsch, Mohammad Al-Gharaibeh, Regine Brandt, Stefan Trogisch, Steffen Heinecke, Susanne Lachmuth, and Svenja Haenzel, for their help and companionship. Amira Apaza Quevedo, Monika Patzsch, and Regine Brandt really gave me a lot of help in my daily life especially at my first PhD year; they made my life much easier in that time. I also appreciated the help from Prof. Helge Bruelheide, who gave me many chances to get to know more excellent scientists. All of these people provide me a very comfortable and good atmosphere to work in the Botanical Garden.

I am really appreciated that Dr. Andreas Gogol-Döring gave me to chance to join the Bioinformatics Unit (BIU) in the Leipzig idiv in my last PhD year. In the Leipzig idiv, I had very good chance to talk and communicate with young scientist. Many thanks to the collegues and friends in idiv, Yvonne Pöschl, Silvio Weging, Irene Bender, Gabriela Martucci do Couto, Wubin Xu, Mengyun Liu, Yuanheng Li for their help and companionship.

I thank all authors/coauthors of my publications and all people who supported this work in some way, Bin Tian, Daniel Renison, Jana Macková, Jing Wang, Laura Morales, Michael Kessler, Paul M. Ramsay, Petr Macek, Silvia C. Gallegos, Susanne Lachmuth, Ricardo Suarez, Romoleroux Katya, and Xinmin Tian. Especially, I want to thank Laura Morales and Susanne Lachmuth for checking the grammar of my dissertation. I am very glad I could collaborate with these people from different universities all over the world.

I also want to thank all the friends in Halle and Leipzig. First, my language tandom partner, Felix Keitel, Clemens Oerding and Shou Li, although my german is not good enough to communicate, but I had good experience learning new language and exchanging different

culture, including music and food. Second, the team of exploring new food and new scenes, Xuefeng Gong, Fang Yin, Zhiqin Pei, Shuli Guo, Yulong Li, and so on, they make weekend life interesting.

I want to thank the financial support of the China Scholarship Council for allowing my stay in Germany. My heartfelt gratitude goes to my family for their love, support, encouragement and for the valuable lessons about overcoming difficult circumstances. Lastly, I would like to express indebtedness and deep sense of gratitude to my husband, Yu Lei, for his love, encouragement and unconditional support.

Appendix

Appendix 1

Supplementary information for this dissertation

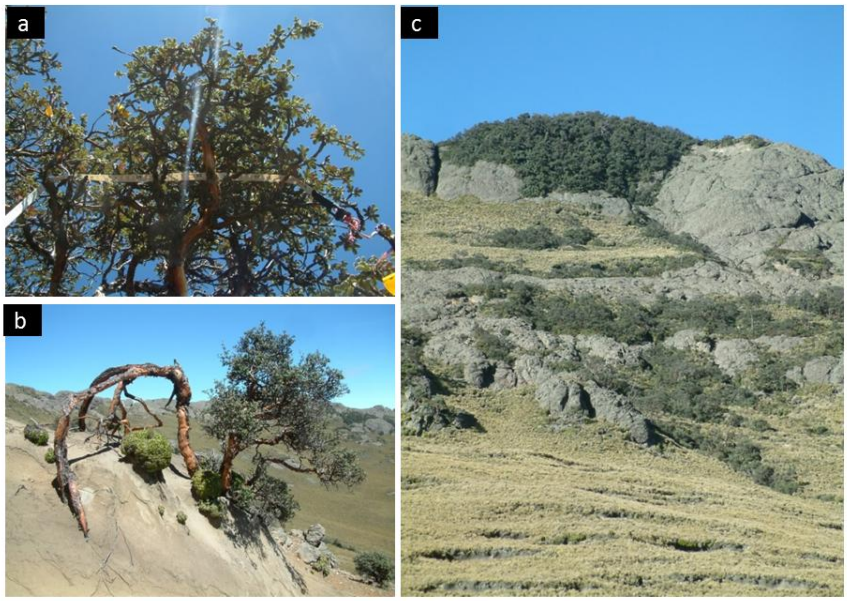


Fig. S1. *Polylepis reticulata* from the studied plot (Photos from Petr Macek).

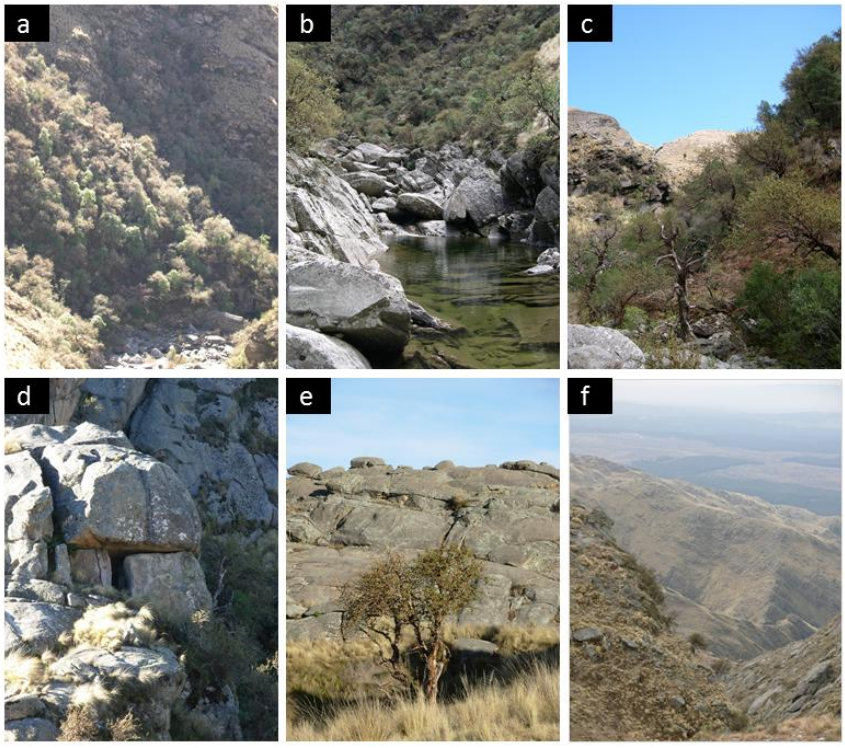


Fig. S2. One conserved forest (a,b,c) and one fragmented forest (d,f,e) of *Polylepis australis* caused by differing human histurbance (Photos from Daniel Renison).

Supplementary infomation for Chapter 2

Table S1 The sampling detail of the 117 individuals from two transects. X and Y represent coordinates measured in meters along or across the transect.

Sample	X	Y	Sample	X	Y	Sample	X	Y	Sample	X	Y
Coarse transect			Fine transect			Fine transect			Fine transect		
R0A	0	0	R1	14	0	R37	16	0	R73	18	0
R0B	0	2	R2	14	0.5	R38	16	0.5	R74	18	0.5
R0C	0	4	R3	14	1	R39	16	1	R75	18	1
R2A	2	0	R4	14	1.5	R40	16	1.5	R76	18	1.5
R2B	2	2	R5	14	2	R41	16	2	R77	18	2
R2C	2	4	R6	14	2.5	R42	16	2.5	R78	18	2.5
R5A	5	0	R7	14	3	R43	16	3	R79	18	3
R5B	5	2	R8	14	3.5	R44	16	3.5	R80	18	3.5
R5C	5	4	R9	14	4	R45	16	4	R81	18	4
R9A	9	0	R10	14.5	0	R46	16.5	0	R73	18	0
R9B	9	2	R11	14.5	0.5	R47	16.5	0.5	R74	18	0.5
R9C	9	4	R12	14.5	1	R48	16.5	1	R75	18	1
R14A	14	0	R13	14.5	1.5	R49	16.5	1.5	R76	18	1.5
R14B	14	2	R14	14.5	2	R50	16.5	2	R77	18	2
R14C	14	4	R15	14.5	2.5	R51	16.5	2.5	R78	18	2.5
R20A	20	0	R16	14.5	3	R52	16.5	3			
R20B	20	2	R17	14.5	3.5	R53	16.5	3.5			
R20C	20	4	R18	14.5	4	R54	16.5	4			
R27A	27	0	R19	15	0	R55	17	0			
R27B	27	2	R20	15	0.5	R56	17	0.5			
R27C	27	4	R21	15	1	R57	17	1			
R35A	35	0	R22	15	1.5	R58	17	1.5			
R35B	35	2	R23	15	2	R59	17	2			
R35C	35	4	R24	15	2.5	R60	17	2.5			
R45A	45	0	R25	15	3	R61	17	3			
R45B	45	2	R26	15	3.5	R62	17	3.5			
R45C	45	4	R27	15	4	R63	17	4			
R57A	57	0	R28	15.5	0	R64	17.5	0			
R57B	57	2	R29	15.5	0.5	R65	17.5	0.5			
R57C	57	4	R30	15.5	1	R66	17.5	1			
R77A	77	0	R31	15.5	1.5	R67	17.5	1.5			
R77B	77	2	R32	15.5	2	R68	17.5	2			
R77C	77	4	R33	15.5	2.5	R69	17.5	2.5			
R100A	100	0	R34	15.5	3	R70	17.5	3			
R100B	100	2	R35	15.5	3.5	R71	17.5	3.5			
R100C	100	4	R36	15.5	4	R72	17.5	4			

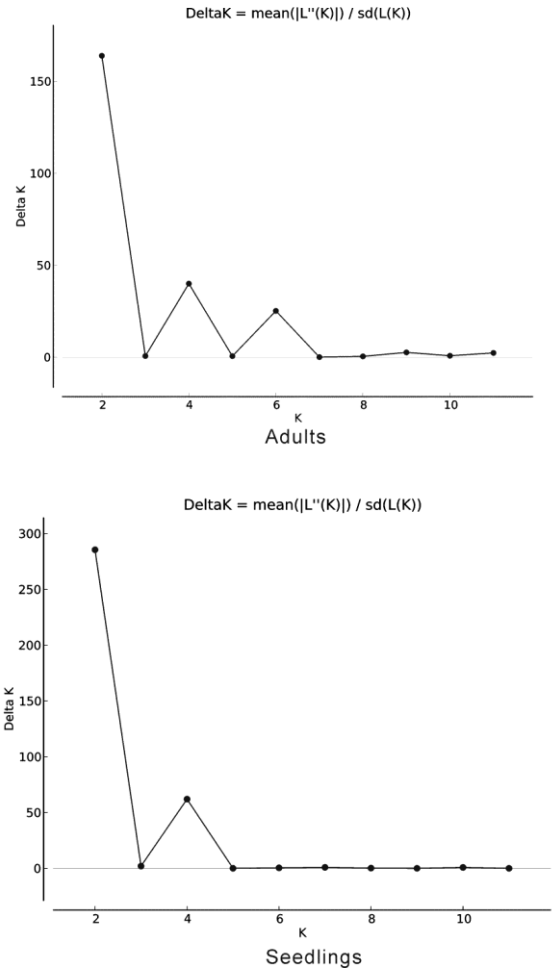
Table S2 The number of trees with flowers or fruits in total of 20 trees from October 2008 to November 2009 in the sampled location.

Date	Total	N(Flowers)	% N(Flowers)	N(Fruits)	% N(Fruits)
10-10-2008	20	13	65	0	0
17-11-2008	20	13	65	2	10
05-01-2009	20	20	100	0	0
14-02-2009	20	11	55	0	0
04-04-2009	20	12	60	2	10
09-05-2009	20	10	50	3	15
22-06-2009	20	10	50	1	5
26-07-2009	20	11	55	1	5
03-10-2009	20	17	85	0	0
22-11-2009	20	15	75	0	0

Supplementary information for Chapter 4

APPENDIX S1 Quantitative characteristics of final peak selection from AFLP fingerprints of *Polylepis tarapacana* with five combinations

	Total	AGC/CTG	AGC/CAT	ACT/CTG	AAG/CAT	AAG/CTG
Number of samples	768	746	754	752	737	745
Number of duplicated individuals		168	205	182	162	234
Duplicated individuals in total		22%	27%	24%	22%	31%
Number of markers present from automatic scoring	1651	319	332	340	324	336
H2 > 0.25, *P* < 0.05	465	56	118	111	107	73



APPENDIX S2 The relationship between DeltaK and K for *Polylepis tarapacana* adults and seedlings, respectively.

Supplementary information for Chapter 5

Table S1 Sampled localities, mtDNA and cpDNA haplotypes, as well as estimates of gene diversity for each population.

Species/populations	Latitude (N)	Longitude (E)	Alt.(m)	*N*	h_{mt}	n_{mt}	H_{mt}	N_{mt}	h_{cp}	n_{cp}	H_{cp}	N_{cp}
A. densa												
1-Cuona XZ	27°54.600'	091°49.050'	3453	8	M1(8)	1	0	0	C36(8)	1	0	0
2-Yadong XZ	27°43.748'	088°58.368'	3500	8	M1(8)	1	0	0	C36(8)	1	0	0
A. spectablis												
3-Nepal	28°57.000'	084°71.000'	3040	11	M3(11)	1	0	0	C2(10) C10(1)	2	0.182	0.024
4-Nepal	27°34.000'	094°43.000'	3450	5	M2(3) M4(2)	2	0.600	0.454	C9(2) C10(3)	2	0.60	0.024
5-Nielamu XZ	32°38.586'	085°56.830'	3540	8	M4(8)	1	0	0	C2(4) C4(2) C26(1) C28(1)	4	0.75	0.059
6-Jilong XZ	28°26.838'	085°34.865'	3025	8	M4(5) M5(3)	2	0.536	0.113	C2(4) C7(1) C16(1) C18(1) C19(1)	5	0.786	0.156
7-Zhangmu XZ	28°02.290'	085°59.140'	2874	11	M4(11)	1	0	0	C2(11)	1	0	0
8-Nepal	29°36.361'	080°59.959'	3042	6	M1(3) M6(3)	2	0.600	0.126	C4(6)	1	0	0
9-Nepal	29°21.400'	081°04.317'	3121	7	M1(7)	1	0	0	C4(7)	1	0	0
10-Nepal	29°24.383'	081°08.183'	2850	6	M1(3) M6(3)	2	0.600	0.126	C4(6)	1	0	0
11-Jilong XZ	28°32.290'	085°13.410'	3390	11	M4(11)	1	0	0	C2(11)	1	0	0
A. pindrow												
12-Nepal	29°24.383'	081°08.183'	2850	10	M1(3) M6(7)	2	0.467	0.099	C2(1) C4(1) C14(5) C35(3)	4	0.711	0.063
A. recurvata												
13-Baiyu SC	31°22.349'	098°53.076'	2979	14	M1(14)	1	0	0	C1(1) C7(13)	2	0.143	0.006
14-Yuangenshan SC	29°08.744'	099°35.113'	3333	10	M1(10)	1	0	0	C5(1) C6(2) C7(5) C8(1) C11(1)	5	0.756	0.079
15-Litang SC	31°29.476'	100°18.185'	3001	10	M1(10)	1	0	0	C6(10)	1	0	0

Followed by Table S1

Species/populations	Latitude (N)	Longitude (E)	Alt.(m)	*N*	h_{mt}	n_{mt}	H_{mt}	N_{mt}	h_{cp}	n_{cp}	H_{cp}	N_{cp}
16-Xinlong SC	30°58.947'	100°05.792'	3068	7	M1(7)	1	0	0	C1(1) C3(1) C6(2) C8(3)	4	0.810	0.047
17-Luhuo SC	31°32.970'	100°42.297'	3179	11	M1(11)	1	0	0	C3(1) C6(3) C8(3) C11(2) C22(1) C24(1)	6	0.873	0.073
18-Seda SC	31°52.885'	100°45.626'	3302	11	M1(11)	1	0	0	C8(1) C12(1) C22(9)	3	0.346	0.021
19-Daofu SC	31°04.524'	100°59.967'	3060	3	M1(3)	1	0	0	C6(3)	1	0	0
20-Danba SC	31°52.067'	101°56.969'	2826	5	M1(5)	1	0	0	C15(2) C16(3)	2	0.600	0.024
21-Maerkang SC	31°54.351'	102°12.375'	3400	9	M1(9)	1	0	0	C11(1) C19(2) C22(6)	3	0.556	0.037
22-Rangtang SC	32°13.001'	100°58.800'	3255	11	M1(11)	1	0	0	C3(1) C8(2) C11(1) C22(6) C31(1)	5	0.709	0.049
A. squamata												
23-Batang SC	30°17.247'	099°30.550'	4220	10	M1(10)	1	0	0	C5(1) C6(2) C8(6) C22(1)	4	0.644	0.031
24-Xiangcheng	29°08.578'	099°55.099'	3375	14	M1(14)	1	0	0	C1(2) C6(1) C8(9) C20(1) C21(1)	5	0.593	0.047
25-Hongyuan SC	31°55.606'	102°39.254'	3600	11	M1(11)	1	0	0	C7(2) C15(9)	2	0.327	0.013
26-Daocheng SC	29°14.438'	100°05.175'	3988	10	M1(10)	1	0	0	C8(9) C23(1)	2	0.200	0.008
27-Banma QH	32°38.874'	100°56.076'	3540	15	M1(15)	1	0	0	C5(1) C8(2) C22(11) C29(1)	4	0.467	0.028
28-Daofu SC	30°40.431'	101°23.648'	3700	18	M1(18)	1	0	0	C6(6) C8(7) C22(4) C31(1)	4	0.726	0.042
29-Xinlong SC	31°07.624'	098°58.964'	3203	9	M1(9)	1	0	0	C5(3) C7(2) C8(3) C20(1)	4	0.806	0.079
30-Litang SC	30°15.857'	100°15.849'	3850	10	M1(10)	1	0	0	C5(1) C6(1) C8(7) C33(1)	4	0.533	0.024
31-Aba SC	32°44.253'	102°05.864'	3784	5	M1(5)	1	0	0	C7(5)	1	0	0
32-Aba SC	32°39.036'	101°34.950'	3201	12	M1(12)	1	0	0	C8(1) C17(1) C22(10)	3	0.318	0.019
A. georgei												
33-Linzhi XZ	29°39.277'	094°19.875'	4150	11	M1(8) M4(3)	2	0.436	0.092	C7(1) C15(10)	2	0.182	0.007
34-Napahai YN	27°55.180'	099°36.208'	3867	7	M1(7)	1	0	0	C5(1) C8(6)	2	0.286	0.012

Followed by Table S1

Species/populations	Latitude (N)	Longitude (E)	Alt.(m)	*N*	h_{mt}	n_{mt}	H_{mt}	N_{mt}	h_{cp}	n_{cp}	H_{cp}	N_{cp}
35-Bamaxueshan YN	28°24.558'	098°58.953'	3792	12	M1(12)	1	0	0	C1(5) C6(2) C7(2) C8(1) C22(1) C34(1)	6	0.818	0.060
36-Jiaozixueshan YN	25°32.283'	102°30.012'	4023	15	M1(15)	1	0	0	C22(14) C32(1)	2	0.133	0.005
37-Jichoushan SC	29°17.495'	101°28.084'	3972	13	M1(13)	1	0	0	C5(2) C7(3) C8(7) C30(1)	4	0.680	0.051
38-Deqin YN	28°00.160'	098°48.869'	3526	9	M1(9)	1	0	0	C8(9)	1	0	0
39-Gongshan YN	27°57.476'	098°43.881'	3540	18	M1(18)	1	0	0	C8(18)	1	0	0
40-Janziwaishan SC	30°00.140'	100°52.127'	4200	9	M1(9)	1	0	0	C6(8) C7(1)	2	0.222	0.029
41-Baoxing SC	30°20.000'	102°25.000'	3200	17	M1(17)	1	0	0	C15(2) C38(8) C39(7)	3	0.632	0.065
42-Yajiang SC	30°00.140'	100°52.000'	4200	8	M1(6) M2(1) M3(1)	3	0.464	0	C5(3) C8(4) C23(1)	4	0.679	0.032
43-Xiaoxuehshan YN	28°19.349'	099°45.063'	3860	6	M1(6)	1	0	0	C8(6)	1	0	0
44-Daxueshan YN	28°34.309'	099°49.829'	4162	10	M1(10)	1	0	0	C8(9) C30(1)	2	0.200	0.008
45-Honglashan XZ	29°16.484'	098°40.337'	4148	13	M1(13)	1	0	0	C1(5) C7(4) C8(2) C34(1) C41(1)	5	0.782	0.048
A. ernestii												
46-Batang SC	29°43.366'	098°47.456'	3400	10	M1(10)	1	0	0	C1(1) C6(1) C7(4) C8(3) C22(1)	5	0.800	0.063
47-Danba SC	31°52.067'	102°23.969'	2826	7	M1(7)	1	0	0	C7(2) C16(1) C26(4)	3	0.667	0.065
48-Kangding SC	29°33.254'	101°19.826'	3199	10	M1(10)	1	0	0	C1(1) C6(5) C7(3) C37(1)	4	0.711	0.076
49-Baoxing SC	27°55.180'	099°36.208'	3867	7	M1(7)	1	0	0	C7(7)	1	0	0

Followed by Table S1

Species/populations	Latitude (N)	Longitude (E)	Alt.(m)	*N*	h_{mt}	n_{mt}	H_{mt}	N_{mt}	h_{cp}	n_{cp}	H_{cp}	N_{cp}
									C1(3) C7(5) C8(5) C11(1) C22(1)			
50-Weixi YN	27°20.368'	099°15.567'	2605	21	M1(21)	1	0	0	C30(6)	6	0.819	0.064
51-Zhongdian YN	27°39.665'	099°53.976'	3128	13	M1(13)	1	0	0	C1(1) C7(5) C8(3) C15(1) C30(3)	5	0.795	0.062
52-Weixi YN	27°09.062'	099°30.382'	3120	7	M1(7)	1	0	0	C13(2) C15(4) C27(1)	3	0.667	0.031
A. fabri												
53-Erlangshan SC	29°51.419'	102°26.945'	2869	11	M1(11)	1	0	0	C22(11)	1	0	0
54-Emeishan SC	29°36.235'	103°29.437'	2879	6	M1(6)	1	0	0	C22(6)	1	0	0
55-Puge SC	27°35.026'	102°25.955'	2893	11	M1(11)	1	0	0	C22(10) C38(1)	2	0.182	0.015
56-Ebian SC	29°13.826'	103°15.756'	3421	12	M1(12)	1	0	0	C22(12)	1	0	0
57-Dayi SC	30°37.762'	103°10.385'	2700	14	M1(14)	1	0	0	C31(2) C39(12)	2	0.264	0.011
58-Baoxing SC	30°26.453'	102°38.085'	2400	7	M4(7)	1	0	0	C22(1) C31(4) C39(1) C40(1)	4	0.714	0.035
59-Gonggashan XZ	29°35.467'	101°53.067'	3400	14	M1(14)	1	0	0	C22(13) C31(1)	2	0.143	0.006
									C8(1) C12(2) C15(5) C22(4)			
60-Muli SC	27°54.716'	101°25.515'	3450	14	M1(14)	1	0	0	C23(1) C30(1)	6	0.813	0.062
A. forrestii												
									C8(4) C12(1) C22(5) C25(1)			
61-Yulongxueshan YN	27°02.347'	100°12.208'	3080	13	M1(13)	1	0	0	C42(2)	5	0.782	0.054
62-Zhongdian YN	27°39.665'	099°53.976'	3128	4	M1(4)	1	0	0	C7(2) C8(1) C15(1)	3	0.833	0.048
63-Napahai YN	27°55.180'	099°36.208'	3867	2	M1(2)	1	0	0	C8(2)	1	0	0
64-Daxueshan YN	28°34.309'	099°49.829'	4162	3	M1(3)	1	0	0	C8(3)	1	0	0
65-Muli SC	27°51.532'	101°32.345'	3764	13	M1(13)	1	0	0	C5(1) C8(7) C22(3) C39(1) C42(1)	5	0.692	0.035
66-Kangding SC	29°56.425'	101°57.745'	3587	10	M1(10)	1	0	0	C8(2) C22(4) C31(4)	3	0.711	0.037

Followed by Table S1

Species/populations	Latitude (N)	Longitude (E)	Alt.(m)	*N*	h_{mt}	n_{mt}	H_{mt}	N_{mt}	h_{cp}	n_{cp}	H_{cp}	N_{cp}
A. nukiangensis												
67-Lushui YN	25°58.347'	098°41.021'	2890	7	M1(7)	1	0	0	C30(7)	1	0	0
68-Lushui YN	25°44.856'	099°03.442'	3200	9	M1(9)	1	0	0	C30(9)	1	0	0
69-Lanping YN	26°27.445'	099°18.834'	2956	2	M1(2)	1	0	0	C7(1) C30(1)	2	1	0.130
70-Ninglang YN	27°52.891'	100°48.609'	2980	13	M1(13)	1	0	0	C7(10) C8(1) C30(2)	3	0.410	0.045
71-Zhongdian YN	27°39.665'	099°53.976'	3128	7	M1(7)	1	0	0	C7(2) C8(3) C30(2)	3	0.762	0.060
72-Gongshan YN	27°36.531'	098°34.551'	3300	11	M1(11)	1	0	0	C8(10) C43(1)	2	0.182	0.015
A. delavayi												
73-Cangshan YN	25°49.378'	100°02.468'	3300	9	M1(9)	1	0	0	C15(1) C30(8)	2	0.222	0.018
74-Motuo XZ	29°25.984'	095°58.673'	3450	8	M1(8)	1	0	0	C15(1) C30(7)	2	0.250	0.021
A. ferrena												
75-Napahai YN	27°55.457'	099°36.985'	3867	6	M1(6)	1	0	0	C8(6)	1	0	0

Abbreviations: N, the number of individuals in each population; n_{mt}, n_{cp}, the number of mitotypes and chrolotypes, respectively; H_{mt} and H_{cp}, gene diversity of mtDNA and cpDNA, respectively; N_{mt} and N_{cp}, nucleotide diversity of mtDNA and cpDNA, respectively; SC, Sichuan; YN, Yunnan; QH, Qinghai; XZ, Xizang.

Table S2 Counts of mitotypes and chlorotypes of 75 populations from 12 species in the QTP and Himalayas.

	Species	*Abies densa*	*Abies spectablis*	*Abies pindrow*	*Abies recurvata*	*Abies squamata*	*Abies georgei*	*Abies ernestii*	*Abies fabri*	*Abies forrestii*	*Abies nukiangensis*	*Abies delavayi*	*Abies ferreana*	Total/Average	Frequency (%)
	Np	2	9	1	10	10	13	7	8	6	6	2	1	75	
	N	16	73	10	91	114	148	75	89	45	49	17	6	733	
Mito-types	Uh	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Hmt	0	0.26	0.47	0	0	0.07	0	0	0	0	0	0	0.07	
	Nmt	0	0.091	0.1	0	0	0.01	0	0	0	0	0	0	0.02	
	M1	16	13	3	91	114	143	75	82	45	49	17	6	654	89
	M2		3				1							4	0.5
	M3		11				1							12	1.6
	M4		37				3		7					47	6.4
	M5		3											3	0.4
	M6		6	7										13	1.8
	Np	2	9	1	10	10	13	7	8	6	6	2	1	75	
	N	16	73	10	91	114	148	75	89	45	49	17	6	733	
Chloro-types	Nh	1	10	4	14	14	14	13	10	10	4	2	1	43	5.9
	Uh	1	3	2	2	6	3	3	1	2	1	0	0	24	3.3
	C1				2	2	10	6	0.02					20	2.7
	C2		40	1										41	5.6
	C3				3									3	0.4
	C4		21	1										22	3

Followed by Table S2

	Species	*Abies densa*	*Abies spectablis*	*Abies pindrow*	*Abies recurvata*	*Abies squamata*	*Abies georgei*	*Abies ernestii*	*Abies fabri*	*Abies forrestii*	*Abies nukiangensis*	*Abies delavayi*	*Abies ferreana*	Total/Average	Frequency (%)
Chloro-types	C5				1	6	6			1				14	1.9
	C6				20	10	10	6						46	6.3
	C7		1		18	9	11	26		2	13			80	11
	C8				10	44	62	11	1	19	14		6	167	23
	C9		2											2	0.3
	C10		4											4	0.5
	C11				5			1						6	0.8
	C12				1				2	1				4	0.5
	C13							2						2	0.3
	C14			5										5	0.7
	C15				2	9	12	5	5	1		2		36	4.9
	C16		1		3			1						5	0.7
	C17					1								1	0.1
	C18		1											1	0.1
	C19		1		2									3	0.4
	C20					2								2	0.3
	C21					1								1	0.1
	C22				22	26	15	2	57	12				134	18
	C23					1	1		1					3	0.4
	C24				1									1	0.1
	C25									1				1	0.1

Followed by Table S2

	Species	*Abies densa*	*Abies spectablis*	*Abies pindrow*	*Abies recurvata*	*Abies squamata*	*Abies georgei*	*Abies ernestii*	*Abies fabri*	*Abies forrestii*	*Abies nukiangensis*	*Abies delavayi*	*Abies ferreana*	Total/Average	Frequency (%)
	C26		1					4						5	0.7
	C27							1						1	0.1
	C28		1											1	0.1
	C29					1								1	0.1
	C30						2	9	1		21	15		48	6.5
	C31				1	1			7	4				13	1.8
	C32						1							1	0.1
Chloro-	C33					1								1	0.1
	C34						2							2	0.3
types	C35			3										3	0.4
	C36	16												16	2.2
	C37							1						1	0.1
	C38						8		1					9	1.2
	C39						7		13	1				21	2.9
	C40								1					1	0.1
	C41						1							1	0.1
	C42									3				3	0.4
	C43										1			1	0.1

Abbreviations: N, the number of individuals in each species; Nh, Number of haplotypes; Hmt, Hcp, genetic diversity of mtDNA and cpDNA, respectively, Nmt, Ncp, Nucleotide diversity of mtDNA and cpDNA, respectively.

Table S3 Pairwise F_{ST} were calculated of mtDNA and cpDNA between the 12 species from the southeastern QTP and Himalayas.

	A. densa	*A. spectabilis*	*A. pindrow*	*A. recurvata*	*A. squamata*	*A. georgei*	*A. ernestii*	*A. fabri*	*A. forrestii*	*A. nukiangensis*	*A. delavayii*	*A. ferreana*
A. densa		0.267	-0.001	0.000	0.000	-0.025	0.000	0.012	0.000	0.000	0.000	0.000
A. spectabilis	**0.693**		**0.394**	**0.425**	**0.458**	**0.439**	**0.399**	**0.326**	**0.342**	**0.350**	**0.271**	**0.211**
A. pindrow	**0.775**	**0.571**		**0.920**	**0.934**	**0.808**	**0.906**	**0.734**	**0.860**	**0.868**	**0.739**	**0.600**
A. recurvata	**0.624**	**0.595**	**0.578**		0.000	0.006	0.000	**0.069**	0.000	0.000	0.000	0.000
A. squamata	**0.705**	**0.670**	**0.667**	**0.045**		0.009	0.000	**0.080**	0.000	0.000	0.000	0.000
A. georgei	**0.686**	**0.663**	**0.655**	**0.060**	**0.010**		0.004	0.015	-0.002	-0.001	-0.023	-0.084
A. ernestii	**0.612**	**0.581**	**0.556**	**0.089**	**0.169**	**0.148**		**0.061**	0.000	0.000	0.000	0.000
A. fabri	**0.801**	**0.757**	**0.762**	**0.212**	**0.211**	**0.232**	**0.421**		0.045	0.047	0.015	-0.046
A. forrestii	**0.770**	**0.713**	**0.699**	**0.089**	**0.031**	**0.045**	**0.253**	**0.109**		0.000	0.000	0.000
A. nukiangensis	**0.724**	**0.680**	**0.656**	**0.161**	**0.168**	**0.137**	**0.111**	**0.423**	**0.232**		0.000	0.000
A. delavayii	**0.940**	**0.769**	**0.809**	**0.379**	**0.410**	**0.378**	**0.379**	**0.590**	**0.475**	**0.182**		0.000
A. ferreana	**1.000**	**0.723**	**0.749**	0.096	0.004	-0.015	**0.186**	**0.349**	0.058	**0.143**	**0.692**	

The upper part is mtDNA, the lower part is cpDNA. Bold number is significant value ($P < 0.05$).

Appendix 2

Curriculum Vitae

Personal data

Name:	Yanling Peng
Date of birth:	17.10.1987
Place of birth:	Hunan
Nationality:	Chinese
Civil status:	Married
Address:	Nordstrasse 39, 04105 Leipzig
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Education

Since March. 2012	Ph.D Martin-Luther-University of Halle / Institute for Biology / Geobotany and Botanical Garden / Working Group Plant Ecology Prof. Dr. Isabell. Hensen
Sep. 2009 – Dec. 2011	Master Lanzhou University / College of Life Science / State Key Laboratory of Grassland Agro-Ecosystem Prof. Dr. Jianquan Liu
Sep. 2005 – Jul. 2009	Basic studies at Lanzhou University

School education

Sep. 2002 – Jul. 2005	Senior high school at Guiyang Sanzhong
Sep. 1999 – Jul. 2002	Junior high school at Guiyang Qizhong
Sep. 1998 – Jul. 1999	Sizhou Center for little
Sep. 1993 – Jul. 1998	Primary school at Cuijiang

Publications of the dissertation

The thesis is based on the following publications, which are referred throughtout the test by their Latin numerals:

1. Peng Y, Macek P, Macková J, Romoleroux K, Hensen I (2015) Clonal diversity and fine-scale genetic structure in a high Andean treeline population *Biotropica* 47(1): 59-65 doi: 10.1111/btp.12175.

2. Peng Y, Morales L, Hensen I, Renison D (2015) Low genetic differentiation in *Polylepis australis* along elevation gradients in Argentina. (Under review) *Austral Ecology*.

3. Peng Y, Lachmuth S, Gallegos S, Kessler M, Ramsay P, Renison D, Suarez R, Hensen I (2015) Pleistocene climatic oscillations rather than recent human disturbance influence genetic diversity in one of the world`s highest treeline species. *American Journal of Botany* 102(10): 1676-1684 doi: 10.3732/ajb.1500131.

4. Peng Y, Tian B, Tian X, Wang J, Hensen I, Liu J (2015) Range expansion during Pleistocene drove morphological radiation of the fir genus (*Abies*, Pinaceae) in the Qinghai-Tibet Plateau and Himalayas. *Botanical Journal of the Linnean Society* 179(3): 444-453 doi: 10.1111/boj.12329.

Other Publications by the author

1. Peng Y, Yin S, Wang J, Tian B, Ren G, Guo Q, Liu J (2012) Phylogeographic analysis of the fir species in southern China suggests complex origin and genetic admixture. *Annals of Forest Science* 69: 409-416.

2. Mao K, Milne R, Zhang L, **Peng Y**, Liu J, Thomas P, Mill R, Renner S (2012) Distribution of living Cuppressaceae reflects the breakup of Pangea. *Proceeding of the National Academy of Science of the United States of America* 109: 7793-7798.

3. Ren G, Abott R, Zhou Y, Zhang L, **Peng Y**, Liu J (2012) Genetic divergence, range expansion and possible homoploid hybrid speciation among pine species in Northeast China. *Heredity* 108: 552-562.

4. Wang J, Abbott RJ, **Peng Y**, Du F, Liu J (2011) Species delimitation and biogeography of two fir species (*Abies*) in central China: cytoplasmic DNA variation. *Heredity* 107: 362-370.

5. Zhang L, **Peng Y**, Ren G, Zhou Y, Li Z, Liu J (2011) Population genetic diversity and species divergence of *Pinus massoniana* and *P. hwangshanensis* at two nucleotide loci. *Chinese Journal of Plant Ecology* 35: 531-536.

Contributions to conferences

1. Peng Y, Lachmuth S, Gallegos S, Kessler M, Ramsay P, Renison D, Suarez R, Hensen I (2014) Pleistocene climatic oscillations rather than recent human disturbance influence genetic diversity in one of the world`s highest treeline species. Talk at Annual Conference of the Society for Tropical Ecology (GTÖ), 25-28.02.2014, Zurich.

2. Peng Y, Tian B, Tian X, Wang J, Hensen I, Liu J (2015) Range expansion during Pleistocene drove morphological radiation of the fir genus (*Abies*, Pinaceae) in the Qinghai-Tibet Plateau and Himalayas. Talk at the international conference on the alpine research, 16-18.01.2013, Lanzhou.

Appendix 3

Erklärung über den persönlichen Anteil an den Publikationen

Study 1

Peng Y., Macek P., Macková J., Romoleroux K., Hensen I. (2015) Clonal diversity and fine-scale genetic structure in a high Andean treeline population *Biotropica* 47(1): 59-65 doi: 10.1111/btp.12175.

Data collection (Field and Lab work): Yanling Peng (20%), Petr Macek (35%), Jana Macková (35%), and Katya Romoleroux (10%)

Data analysis: Yanling Peng (100%)

Writing: Yanling Peng (90%), Isabell Hensen (10%)

Corrections by Isabell Hensen, Petr Macek, Jana Macková.

Study 2

Peng Y., Morales L., Hensen I., Renison D. (2015) Low genetic differentiation in *Polylepis australis* along elevation gradients in Argentina. *Austral Ecology* (Under review).

Data collection (Field and Lab work): Yanling Peng (30%), Daniel Renison (60%), Laura Morales (10%)

Data analysis: Yanling Peng (100%)

Writing: Yanling Peng (90%), Daniel Renison (10%)

Corrections by Isabell Hensen, Laura Morales, Daniel Renison.

Study 3

Peng Y., Lachmuth S., Gallegos S., Kessler M., Ramsay P., Renison D., Suarez R., Hensen I. (2015) Pleistocene climatic oscillations rather than recent human disturbance influence genetic diversity in one of the world`s highest treeline species. *American Journal of Botany* 102(10): 1676-1684 doi: 10.3732/ajb.1500131.

Data collection (Field and Lab work): Yanling Peng (30%), Silvia C. Gallegos (10%), Daniel Renison and Ricardo Suarez (30%), Isabell Hensen (30%).

Data analysis: Yanling Peng (95%), Susanne Lachmuth (5%)

Writing: Yanling Peng (90%), Isabell Hensen (10%)

Corrections by all coauthors.

Study 4

Peng Y., Tian B., Tian X., Wang J., Hensen I., Liu J. (2015) Range expansion during

Pleistocene drove morphological radiation of the fir genus (*Abies*, Pinaceae) in the Qinghai-Tibet Plateau and Himalayas. *Botanical Journal of the Linnean Society* 179(3): 444-453 doi: 10.1111/boj.12329.

Data collection (Field and Lab work): Yanling Peng (40%), Bin Tian (40%), Xinmin Tian (10%), Jing Wang (10%)

Data analysis: Yanling Peng (100%)

Writing: Yanling Peng (80%), Jianquan Liu (10%), Isabell Hensen (10%)

Corrections by Jianquan Liu and Isabell Hensen.

Halle (Saale), den

Unterschrift.....

Eigenständigkeitserklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel „The potential genetic responses to environmental changes in forest trees: case studies at multiple spatial scales“ bisher weder der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den
Unterschrift:…….....