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

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ABSTRACT

Clonal propagation becomes more abundant with increasing altitudes 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 m × 100 m (coarse scale) and 4 m × 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. The studied *P. reticulata* population showed pronounced FSGS (Sp = 0.012 at the genet level, a statistic reflecting declining pairwise kinship with distance) revealed restricted gene dispersal, 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. The findings highlights that clonal propagation has affected FSGS within a spatial distance of 3 m for this species.

Abstract in Spanish is available in the online version of this article.

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

At higher altitudes alpine plants experience harsher environmental conditions, 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 having 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 attributed to a number of advantages associated with clonal propagation. First, clonal propagation allows species to survive over long periods of significant climatic oscillation (Honnay & Bossuyt 2005, de Witte *et al.* 2012). Second, 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). Third, 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 have shown that FSGS commonly results from the joint effects of seed and pollen mediated gene flow, as well as, in the case of clonal plants, the pattern of clonal propagation (Vekemans & Hardy 2004, Chung *et al.* 2006). On the one hand, if pollen is dispersed more widely than seeds, such as in plants with gravity-dispersed seeds and wind pollen dispersal, SGS will be more influenced by dispersal of seed than pollen (Sebbenn *et al.* 2011, Wang *et al.* 2011). On the other hand, FSGS can be

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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). 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 quantifying the effects of clonal propagation on FSGS have yet to be conducted. Here, we present a case study of one population of *P. reticulata* in which we use Amplified Fragment Length Polymorphism (AFLP) to study the effects of clonal propagation on genetic diversity and FSGS of one of the world's highest treeline species. We aimed to determine: (1) the clonal and genetic diversity at both larger and smaller scales, and (2) the contribution of clonal propagation and restricted gene dispersal 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 limited dispersal (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 to extinction (Romoleroux & Pitman 2004).

SAMPLING PROCEDURE.—We sampled a population of *P. reticulata* located in the 'Reserva de Producción Faunística Chimborazo' of Ecuador (1.542 S, 78.885 W) 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, we assessed the clonal diversity and genetic structure of one of the largest patches (160 m × 12 m) by collecting leaf samples from one fine transect and one coarse transect, with the fine transect being located within the coarse one (Table S1; Fig. 1). From October 2008 to November 2009 we also observed how many of 20 marked trees in the sampled location had flowers or fruits (Table S2). The most recently formed leaves were collected in 2009, and leaf surfaces were thoroughly cleaned with tissue paper before being stored in bags with silica-gel in order to reduce contamination by epiphytic algae and fungi.

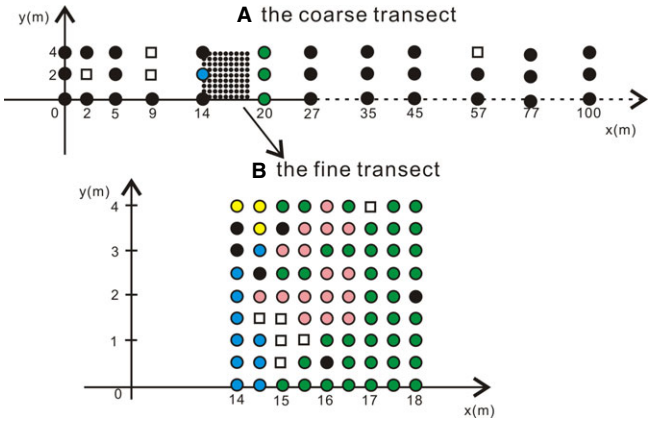

FIGURE 1. The distributions of 38 genets recovered across both transects: (A) The distribution of 30 genets (94.0% in total) from the coarse transect; (B) The distribution of 10 genets recovered from 75 ramets (13.3% in total). The white square represents the missing samples caused by problems with PCR;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

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 collected at each interval (middle, left and right; with ~2 m distance between sampled individuals fulfilling the given parameters). 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 m × 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 to cover a wide range of geographical distances between sampled individuals while minimizing the total number of required samples.

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′-AATTGGTACGCAGTCT AC-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 pre-amplified 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)). Pre-amplification 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 pre-amplification primers, and 4 μL of the ligation product with the following temperature profile: 5 min initial denaturation at 94°C, 20 cycles of 20 sec denaturation at 94°C, 30 sec annealing at 56°C, and 120 sec elongation at 72°C.

The pre-amplification 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 sec denaturation at 94°C, 30 sec annealing at 65°C (decreasing by 1°C per cycle), 120 sec elongation at 72°C, followed by 4 sec 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.—Due to a failed PCR amplification of some samples, 107 of the 117 sampled individuals were analyzed for the study (Table 1; 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 percent.

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 (v. 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 analog 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 corre-

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.

sponding 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).—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 (*He*) and band richness (Br) for the coarse transect and the fine transect were 43.2 percent and 35.5 percent, 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).

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 positive F_{ij} value close to the 10 m, and only one significant negative 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 ($R^2 = 0.0827$, $P < 0.05$, Fig. 3).

DISCUSSION

We found that the clonal diversity of the study population was affected by both sample size and spatial distance. We also found that clonal propagation influenced the FSGS, but mainly at the finer scale (4 m × 4 m). The low genetic diversity values for *P. reticulata* across both transects may be caused by reduced fitness over time, perhaps due to increased levels of selfing and inbreeding depression, or genetic drift due to the small popula-

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

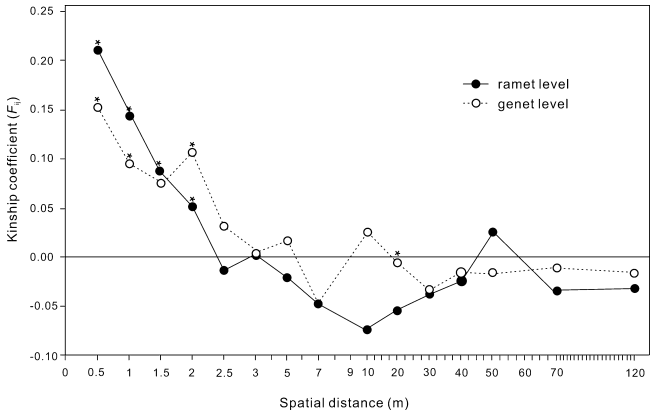

FIGURE 2. Spatial autocorrelation of average kinship coefficient (F_{ij}) over all loci for the whole transect at the ramet level and among-genet level, respectively. '*' indicates kinship coefficient significantly different from zero ($P < 0.05$).

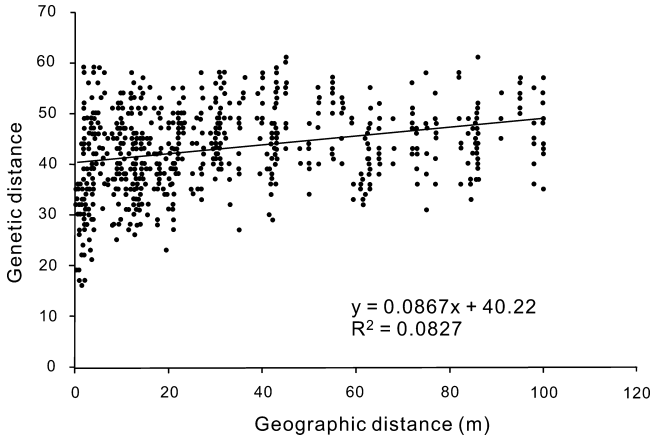

FIGURE 3. Correlation between genetic and geographic distance matrices of studied *P. reticulata* populations along the whole transect (Mantel test).

tion sizes. The pronounced FSGS (*Sp* = 0.012 at the genet level) indicates limited gene flow dispersed 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 of *P. reticulata* revealed that clonal diversity is a function of both sample size and the spatial scale of the sampling area—a result similar to that observed in 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 m × 0.3 m, Reisch *et al.* 2007) or *Rhododendron ferrugineum* (0.08, plot 10 m × 20 m, Escaravage *et al.* 1998). Given genotypic diversity in some self-compatible species (*e.g.*, *Decodon verticillatus*, Eckert 2000, *Zostera mariana*, Reusch 2001) is positively associated with outcrossing rates at a local scale—that is, among neighboring patches within population—the contrasting 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 (*He*) (0.13) at the coarse scale, and the PLP value (35.5%) and *He* (0.09) at the fine scale detected in *P. reticulata,* are similar to those reported for *P. multijuga* (PLP, 54.9%; *He*, 0.13; Quinteros-Casaverde *et al.* 2012) and *P. incana* (PLP, 44.8%; *He*, 0.145; Hensen *et al.* 2012) but much lower than that reported for other *Polylepis spp.* (see Gareca *et al.* 2013). In addition, the *He* found here was expectedly lower than that reported for other species with similar life histories measured using dominant markers (*He* = 0.25–0.27, Nybom 2004). The low genetic diversity here seems consistent with several features of *P. reticulata*. 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). For the coarse transect, the population may have experienced genetic drift due to the small population size (*e.g.*, Wesche *et al.* 2006). It may be that the prolonged lifespan of *Polylepis* individuals—we assume that the longevity of the species could be up to hundreds of years (Suaréz *et al.* 2008, Solíz *et al.* 2009)—allows this species to persist despite these small population sizes.

CAUSES OF FINE-SCALE GENETIC STRUCTURE.—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 of pairs between different genets within a 3 m range (Fig. 2). This can be attributed to the extensive clonal propagation within this spatial range. Further support is 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 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).

The pronounced FSGS found at the genet level (*Sp* = 0.012) may suggest restricted gene dispersal of *P. reticulata*. The *Sp* value we estimated is similar to that reported in predominantly wind-pollinated outcrossing tree species, *e.g., Milicia excels* (maximum *Sp* = 0.006, Bizoux *et al.* 2009), *Castanopsis sclerophylla* (*Sp* = 0.0029-0.0152, Wang *et al.* 2012), and low-elevation stands of *Nothofagus pumilio* (*Sp* = 0.003-0.006, Mathiasen & Premoli 2013). The *Sp* value is also within the range of species with wind pollen dis-

persal (Sp = 0.0054) to species with gravity-dispersed seeds (Sp = 0.0281) (reviewed in Vekemans & Hardy 2004). The pronounced FSGS here is more likely to have resulted from limited gene dispersal (Vekemans & Hardy 2004, Chung *et al.* 2006). It is unknown if this is due to limited seed or pollen dispersal. However, pollen dispersal is considered more extensive in plants with wind-dispersed pollen (*e.g.*, Wang *et al.* 2011), and populations occurred in the vicinity of our focal one, *e.g.*, approximately 1 km to the north (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, and 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). Furthermore, 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 but limited seed dispersal in this population.

An improved understanding of FSGS will enable the development of more effective conservation measures for plant species (Chung *et al.* 2006). In *P. reticulata* the FSGS occurred until the 3 m interval; to optimize future sampling designs and avoid the collection of clone mates in *ex situ* conservation measures we suggest only individuals occurring > 3 m apart should be collected. We hope our study provides a template for evaluating FSGS and its implications in other *Polylepis* populations.

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SUPPORTING INFORMATION

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

Table S1. *The sampling detail of the 117 individuals from two transects.*

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

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