

GENETIC STRUCTURE OF FRAGMENTED POLYLEPIS MULTIJUGA PLIGE (ROSACEAE) FORESTS

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Submitted to the Graduate Faculty of the College of Science and Engineering
Texas Christian University
in partial fulfillment of the requirements
for the degree of
Master of Science
August 2010

ACKNOWLEDGEMENTS

I would like to thank Dr. Dean Williams for his guidance and support throughout this entire process from sample collection through completing my publications. I would also like to thank my graduate committee members, Dr. Amanda Hale, Dr. Matt Chumchal, and Mrs. Tamie Morgan for their help and valuable suggestions. I am grateful to Dr. Tony Burges for his guidance and encouragement during my graduate career.

Special thanks to our collaborators for help with all aspects of this study: Cesar Flores Negrón, Jhon Panduro Cometivos, and Karina Pinasco Vela from the Non Governmental Organization Amazonicos por la Amazonia. Thanks also to Mercedes Flores Pimentel, director of the “MOL” herbarium; Keri McNew, Tiana Franklin, and John Janovec from the Botanical Research Institute of Texas; and the Biology and the Environmental Science Departments at TCU for providing me with stipends which allowed me to complete my research.

My special thanks to all my assistants for their commitment to the project. I would especially like to thank my family for always providing me with inspiration and encouraging me to follow my dreams.

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Introduction

Habitat fragmentation is the conversion of a continuous habitat into fragments of different sizes (Laurance 2005; Lindenmayer and Fischer 2006). Although habitat fragmentation is mainly caused by human activities such as logging, conversion of forests into agricultural fields and cattle pastures, and urbanization, it can also be caused by natural processes such as fire (Laurance 2005; Lindenmayer and Fischer 2006). Forest fragments are often surrounded by a landscape of inhospitable terrain that is predicted to decrease gene flow between once continuous habitat patches resulting in a loss of genetic variability within fragments (Young and Clark 2000; Jump and Pañuelas 2006; Aguilar et al. 2008). Low genetic diversity is predicted to increase the chances of inbreeding depression and decrease the potential for adaptation to changing environmental conditions (Frankham et al. 2007).

The genetic consequences of population fragmentation depend critically upon gene flow and standing levels of genetic diversity. Some recent reviews emphasize that tree populations may be resilient to the effects of habitat fragmentation due to high levels of existing genetic diversity within populations and evidence that long-distance dispersal of pollen, even in fragmented habitats, is high in many tree species (Hamrick 2004; Kramer et al. 2008; Montoya et al. 2008). Nevertheless, Sork and Smouse (2006) stress that the extent of resilience to fragmentation is determined by both the amount of gene flow and the diversity of the pollen pool from which immigration is drawn. Furthermore, several reviews have found that common outcrossing species such as most wind-pollinated trees have greater losses of genetic diversity associated with reduced population sizes than rare or self compatible plants (Honey and Jacquemyn 2007; Aguilar et al. 2008). In contrast to some earlier studies(e.g., insert ref here), Jump and Pañuelas (2006), Kettle et al. (2007), and Dubreuil et al. (2010) found that forest

fragmentation can reduce genetic diversity even in widespread, wind-pollinated trees. The lack of the predicted decrease in genetic diversity found in many 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; Dubreuil et al. 2010).

The genus *Polylepis* from the family Rosaceae (Ruiz and Pavon 1794) comprises 28 species and inhabits the High Tropical Andes (Simpson 1979; Schmidt-Lebuhn et al. 2006). *Polylepis* woodlands are the highest forests in the world (2500 m – 5000 m) and *Polylepis* are adapted for living at high altitudes (Purcell et al. 2004). These woodlands are important habitats for endemic páramo-puna birds and they provide local human communities with firewood and timber (Vuilleumier and Monasterio 1986; Fjeldså 1993 Purcell et al. 2004; Loyd et al. 2008). *Polylepis* forests are currently highly fragmented as a result of human activities such as clearing land for pasture and harvesting the trees for firewood and timber (Fjeldså 2002; Renison et al. 2002, Teich et al. 2005; Renison et al. 2006). Based on niche modeling it is estimated that *Polylepis* forests have been reduced by up to 98% (Fjeldså and Kessler 1996; however, fossil pollen studies suggest that *Polylepis* forests have always been fragmented to some degree (Gosling et al. 2009). Because of current anthropogenic activities and the predicted effects of climate change, these forests were declared “vulnerable” by the IUCN in 2008 and have been listed as one of the most endangered forest types in the world (Seltmann et al. 2009a).

The most primitive member of this genus is *Polylepis multijuga* Pilg, an endemic tree of the northern Andes of Peru that inhabits the highlands of San Martin, La Libertad, Amazonas and Cajamarca (AMPA 2008; IUCN 2008; Leon et al. 2006). Its ecological distribution ranges from 2,500 to 4,000 meters above sea level and encompasses very humid montane forest (*Bosque Muy Húmedo Montano*), wet montane forest (*Bosque Pluvial Montano*), and Páramo (León et al.

2006). *P. multijuga* is a wind-pollinated and wind-dispersed tree (Schmidt-Lebuhn et al. 2006, 2007) and it is currently restricted to fragmented populations due to recent habitat loss caused mainly by anthropogenic actions (Mendoza and León 2006). It is not known, however, to what extent fragmentation may have been caused by humans in the distant past or whether other factors such as microhabitat requirements of the tree might be contributing to its fragmented distribution. *P. multijuga*'s range has been inhabited since pre-Inca times by the Chachapoya people (AD 750–800) and is currently inhabited by Peruvians.

Polylepis multijuga has not been well studied and most of our knowledge is limited to the species description (Macbride 1938), distribution, and recognized threats to its persistence (León et al. 2006). Additional investigation is needed to determine if ongoing habitat loss and fragmentation of populations is affecting the genetic diversity of this species. The goal of this project was to characterize genetic diversity within isolated fragments of *P. multijuga* and determine the degree of gene flow among isolated fragments.

Materials and Methods

Sampling and Study Sites

I sampled four sites located on the eastern slope of the Andes and separated by mountain chains along two Departments (Cajamarca and San Martin) in Northern Peru (Table 1). Three sites were located in San Martin Province of Mariscal Cáceres, and within the Conservation Concession of Alto Huayabamba (CAAH), managed by the NGO Amazonicos por la Amazonia, called “Laguna Huayabamba,” “Lihui,” and “Tragadero.” The fourth site was located in Cajamarca Province of Celendín District of Bella Aurora, and within the property of the Gevara Family, called “Los Gevaras.” Sites were separated by 11 – 79 km (Fig. 1). I collected tissue samples (young leaflets) from 371 trees that were flowering over the course of six weeks

in 2009 (Table 1). Leaflets were dried in small bags containing silica gel. When possible, I collected 25 samples at the edge of the forests and 25 samples inside the forests, with a minimum distance of at least 5 meters between trees.

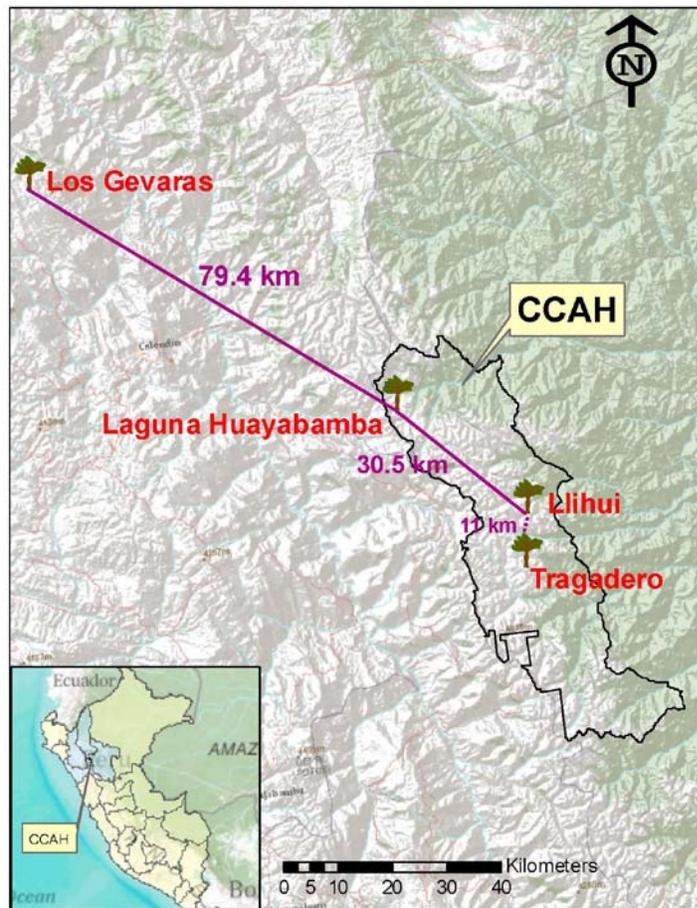


Figure 1 Location map of sampled *Polylepis multijuga* forest fragments.

Laguna Huayabamba is represented by a transitional gradient between grasses at the highest altitude (> 4,000 m) and some scattered trees of *Polylepis* surrounding a dry lake in a deep depression with a very steep slope (Appendix 1). In this area, there are some archeological remains of the Chachapoya culture such as the Vira Vira fortress from where I could see the

Huayabamba Lake. A series of platforms, used in the past for agriculture but now used for cattle ranching, surrounds this lake. The major river in this area is the Huabayacu River, which runs from west to east toward the Amazon Basin. At approximately 3,060 m the presence of *P. multijuga* is more evident. I was able to find two locations with more than one hundred individuals at one site and ~1,000 individuals at the other site. The first site was called “ladera derecha del Huabayacu” (LDH) or “the right slope of the Huabayacu” in English. The second site was called “ladera izquierda del Huabayacu” (LIH) or “the left slope of the Huabayacu” in English. Finally, the third site was called “Laguna Seca” or “Dry Lake” in English.

Lihui is a U-shaped valley covered by a large wetland fed by small lakes and waterfalls which fall in a narrow stream (Appendix 2). The predominant vegetation of this area is high altitude grasses and mosses that cover the ground. Huge boulders were also scattered across the post-glacier terrain. Some pre-Columbian terrace lay in this area. In this area I found one small *P. multijuga* stand, I called this site “Lihui Mountain” (YM). In the transition to the lower areas a big patch of Lihui (a bamboo-like plant) appeared, and the U-shaped valley turned into a deeper canyon. At the end of this vegetation formation a patch of *P. multijuga* was found along the stream that comes from the highlands. This riparian forest seems to have been subject to deforestation and burning with the purpose of growing grasses suitable for cattle. I called this site “Lihui Riparian” (YR).

Tragadero is located on the eastern slope of the Andes. The landscape of this area is very similar to Lihui but wetter. This area received the name of Tragadero or “the swallower” in English because its curious topography is characterized by hundreds of caves created by the erosive power of the water that, when covered with the natural pastures, are an easy trap for a distracted walker. In this area *P. multijuga* stands grow like small aggregations (of at least 25 individuals) in a post-glacier landscape characterized by tall moraines, tarns or post-glacier lakes,

and deep ravines. I chose three sites to sample there (Appendix 3). The first stand was a *Polylepis* cluster (TLE) that was surrounding a lake called Totora. The second stand (TMR) was located at the right part of a big crater pass the big moraine over the mountain slope. The third stand (TDR) was located inside a deep ravine along a narrow stream. This ravine was located before the big moraine, and the stream comes out into the second crater. In contrast to the other sampled stands, I found evidence for natural regeneration (seedlings) of *Polylepis* in the ravine stand.

Los Gevaras was characterized by a high altitude wetland, but in contrast to the other sites, this wetland is being used by people to grow high altitude crops such as potato (*Solanum tuberosum*) and occa (*Oxalis tuberosa*) (Appendix 4). No cattle ranching occur in this area. *P. multijuga* grows on a slope in this area in a fragment with more than 1000 individuals.

DNA was extracted with the IBI Plant Genomic Mini Kit (MIDSCI) following the manufacturer's instructions. AFLPs were amplified using a modified version of Vos et al. (1995). Extracted genomic DNA was double-digested with the restriction enzymes *MseI* and *EcoRI* (New England Biolabs), and the ends of the resulting fragments were ligated to adapter sequences serving as primer binding sites. Restriction and ligation were performed for four hours at 37°C in an 11 µl volume containing 1 unit of *MseI*, 5 units of *EcoRI*, 1 unit of T4 DNA ligase (New England Biolabs), 1 X T4 DNA ligase buffer (New England Biolabs), 0.05 mM NaCl, 0.05 g/l BSA, 5 pmol of *EcoRI*-adapter, 50 pmol *MseI*-adapter, and 5.5 µl DNA extract. The ligation product was diluted with 90 µl of sterile water and then pre-amplified with the primer combination *EcoRI*+A (5'-ACTGCGTACCAATTC+A-3) /*MseI*+C (5'-GATGAGTCCTGAGTAA+C-3). Pre-amplification was performed in a 20 µl volume containing 0.5 U Taq DNA Polymerase (Promega), 1 X PCR reaction buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 5 pmol of each primer, 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.

Table 1 Collection sites for *Polylepis multijuga* in Peru.

Site	Fragment	Lat.-Long.	Elevation (m a.s.l.)	No. Individuals
Laguna Huayabamba (LH)	LDH	77°43'9.592"W 6°59'35.518"S	3,100	57
	LIH	77°42'49.982"W 6°59'3.831"S	3,200	59
	LS	77°44'18.773"W 6°59'34.702"S	3,400	11
Llihui (Y)	YM	77°35'0.77"W 7°11'36.693"S	3,300	17
	YR	77°31'30.453"W 7°10'49.209"S	3,060	50
Tragadero (T)	TMR	77°32'20.912"W 7°16'4.857"S	3,260	30
	TDR	77°32'31.967"W 7°16'21.646"S	3,240	31
	TLE	77°31'34.507"W 7°16'2.936"S	3,200	51
Los Gevaras	LG	78°17'35.889"W 6°34'40.301"S	4,000	65

Amplified Fragment Length Polymorphisms (AFLP)

The pre-amplification product was diluted ten times with sterile water. Selective amplification was carried out in a 20 µl volume containing 0.5 U Taq DNA Polymerase, 1X PCR reaction buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 5 pmol *Mse*I selective primer, 5 pmol

fluorescently labeled *EcoRI* selective primer, and 3 μ l 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 20 cycles of 20 s denaturation at 94°C, 30 s annealing at 56°C and 120 s elongation at 72°C (increasing by 4°C per cycle). For the selective amplification, 17 different primer combinations were tested on a small number of samples for their level of variability. Two of them, Eco+ACA (5-GACTGCGTACCAATTC+ACG-3) / Mse+CCC(5-GATGAGTCCTGAGTAA+CCC-3) and Eco+ACT (E38, 5-GACTGCGTACCAATTC+ACT-3) / Mse+CAC (5-GATGAGTCCTGAGTAA+CAC-3), were chosen for fingerprinting all samples. Selective amplification products were diluted with 200 μ l water and 0.5 μ l was then added to 10 μ l HIDI formamide and 0.1 μ l of LIZ-500 size standard. Fragments were separated on an ABI 3130 Genetic Analyzer (Applied Biosystems).

Raw AFLP fragment data were sized using GeneMapper v 4.0 (Applied Biosystems). The raw phenotype table was exported to AFLPScore (Whitlock et al. 2008). A binary data matrix was produced scoring fragments between 100 and 500 bp of length as present (1) or absent (0). To test for reproducibility, I re-extracted DNA, amplified, and analyzed 48 samples twice.

Statistical Analyses

I calculated the unbiased heterozygosity of each AFLP fragment using the method of Lynch and Milligan (1994) for dominant markers in GenAEx v. 6.3 (Peakall and Smouse 2006). I conducted an analysis of molecular variance (AMOVA) in GenAEx using the F_{ST} analog PhiPT. I also calculated all pair-wise PhiPT values between fragments and tested their significance with 1,000 permutations. I tested for an isolation-by-distance pattern between fragments by correlating the pair-wise genetic distances (PhiPT) between fragments with geographic distance using a Mantel test in GenAEx. I analyzed the relationship between individual pair-wise genetic similarity and geographic distance using the spatial autocorrelation method in GenAEx v 6.3.

This method uses a distance measure between individuals that consists of a tally of differences between their AFLP profiles (Huff et al. 1993). The autocorrelation coefficient, r , is a measure of the genetic similarity between individuals within a defined distance class. The significance of r was determined by 1,000 random permutations of all individuals among distance classes to set the upper and lower 95% confidence limits around $r = 0$. I also bootstrapped r values within each distance class 1,000 times to set the 95% confidence intervals around r . These bootstrapped confidence intervals also provide a graphical test of significance among r values between fragments. I then calculated a pooled estimate of r across fragments using the method described by Peakall et al. (2003). Correlograms are presented with variable sized distance classes to maximize sample sizes within categories while determining the extent of short-distance autocorrelation. I also calculated all pair-wise distances between all individuals and tested whether the average genetic distance among individuals within a fragment was different from what would be expected if individuals were distributed among fragments at random by 1,000 permutations of individuals between fragments. Differences among fragments for this measure were determined by comparing 95% bootstrapped values around the mean genetic distance within fragments. If bootstrap values did not overlap between fragments then they were considered significantly different.

Results

Genetic Variation

The two primer combinations yielded 146 and 136 scorable fragments, respectively. Among the 282 fragments, 95.6% were polymorphic, although 46% of these fragments occurred at a frequency of less than 5%. The overall genetic diversity (H_e) was 0.131 (± 0.01 SE). The

mean genetic diversity (H_e) within forest fragments ranged from 0.098 for the TDR fragment to 0.127 for the TLE fragment (Fig. 2).

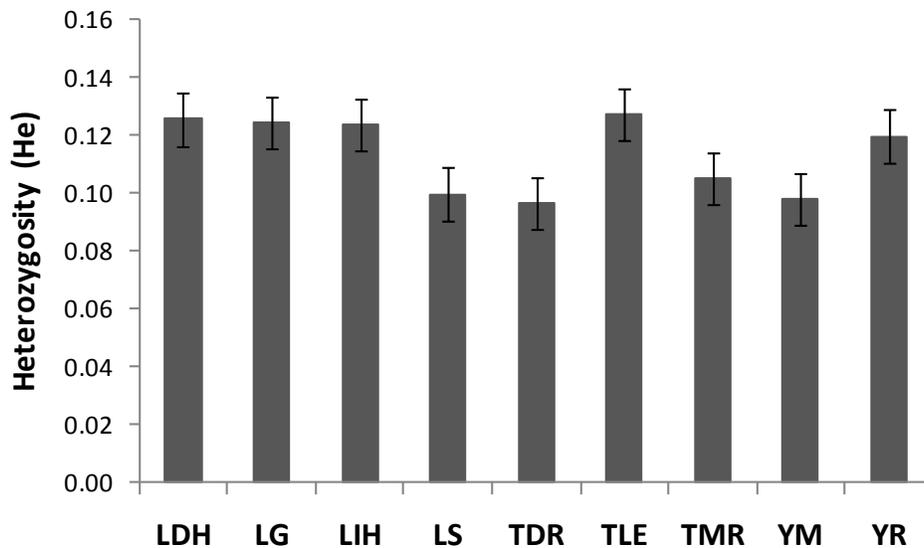


Figure 2 Average heterozygosity (\pm SE) for each *Polylepis multijuga* forest fragment.

AMOVA revealed that 14% of the genetic variance was partitioned among the forest fragments and 87% was found within fragments ($P = 0.001$ in both cases). Pair-wise PhiPT values between fragments ranged from 0.036 – 0.487 (Table 2). All but two pair-wise comparisons between fragments were significantly different (Table 2). The TDR fragment was the most differentiated of all fragments with an average pair-wise PhiPT value of 0.284. A Principal Component Analysis of pair-wise PhiPT values also indicated that TDR was the most divergent fragment (Fig. 3). Divergence among fragments was not related to geographical proximity. Fragments located close together often had higher PhiTP values than when they were compared to more distant fragments (Table 2 and Fig. 3). There was no pattern of isolation-by-distance between fragments (Mantel test, $r = -0.19$, $P = 0.14$; Fig. 4). Average pair-wise individual genetic distances within all fragments were significantly lower than expected if individuals were distributed at random across fragments ($P = 0.001$ in all cases). Four of the fragments (LS, TDR,

TMR, and YM) had significantly lower genetic distances between individuals than the other fragments. TDR had the lowest average genetic distance between individuals. Bootstrapped 95% confidence intervals around TDR only overlapped with YM (Fig. 4).

Table 2 Pair-wise genetic distances between *Polylepis multijuga* forest fragments. PhiPT values are below diagonal and P values are shown above the diagonal.

	LDH	LG	LIH	LS	TDR	TLE	TMR	YM	YR
LDH	0.000	0.001	0.006	0.058	0.001	0.001	0.001	0.001	0.008
LG	0.086	0.000	0.001	0.002	0.001	0.001	0.001	0.004	0.001
LIH	0.056	0.064	0.000	0.001	0.001	0.001	0.001	0.005	0.012
LS	0.049	0.173	0.166	0.000	0.001	0.001	0.061	0.001	0.004
TDR	0.327	0.231	0.210	0.487	0.000	0.001	0.001	0.001	0.001
TLE	0.177	0.117	0.084	0.295	0.109	0.000	0.001	0.002	0.001
TMR	0.095	0.178	0.186	0.050	0.446	0.273	0.000	0.001	0.001
YM	0.175	0.109	0.086	0.345	0.188	0.090	0.320	0.000	0.006
YR	0.044	0.067	0.036	0.131	0.271	0.126	0.141	0.109	0.000

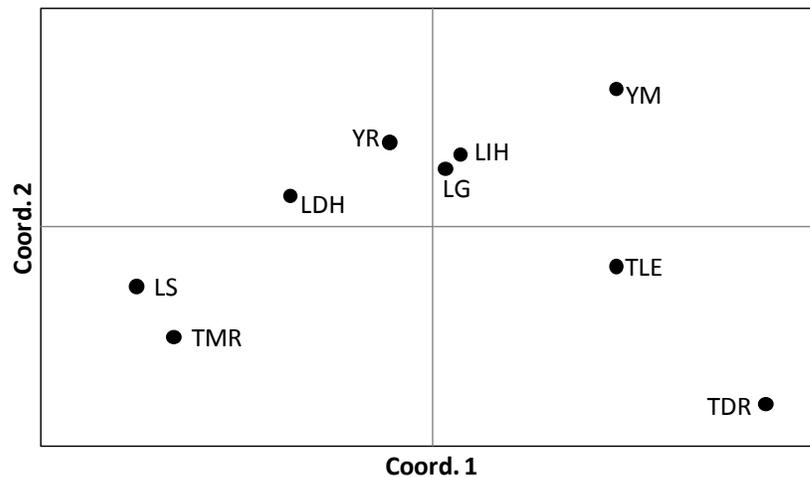


Figure 3 Principal components analysis of pair-wise genetic distances (PhiPT) between *Polylepis multijuga* forest fragments. The first two PCA axes account for 79% of the variation.

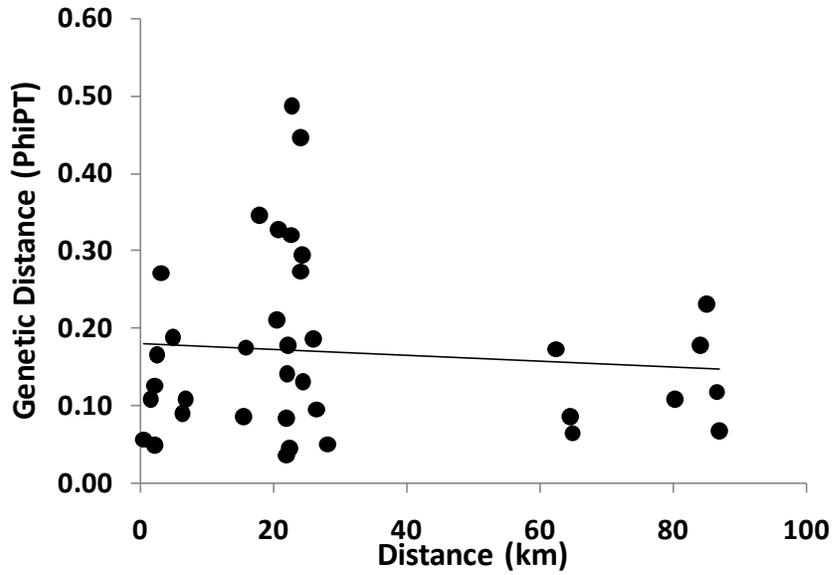


Figure 4 Correlation between geographic distance (km) and genetic distance (PhiPT) for forest fragments of *Polylepis multijuga*.

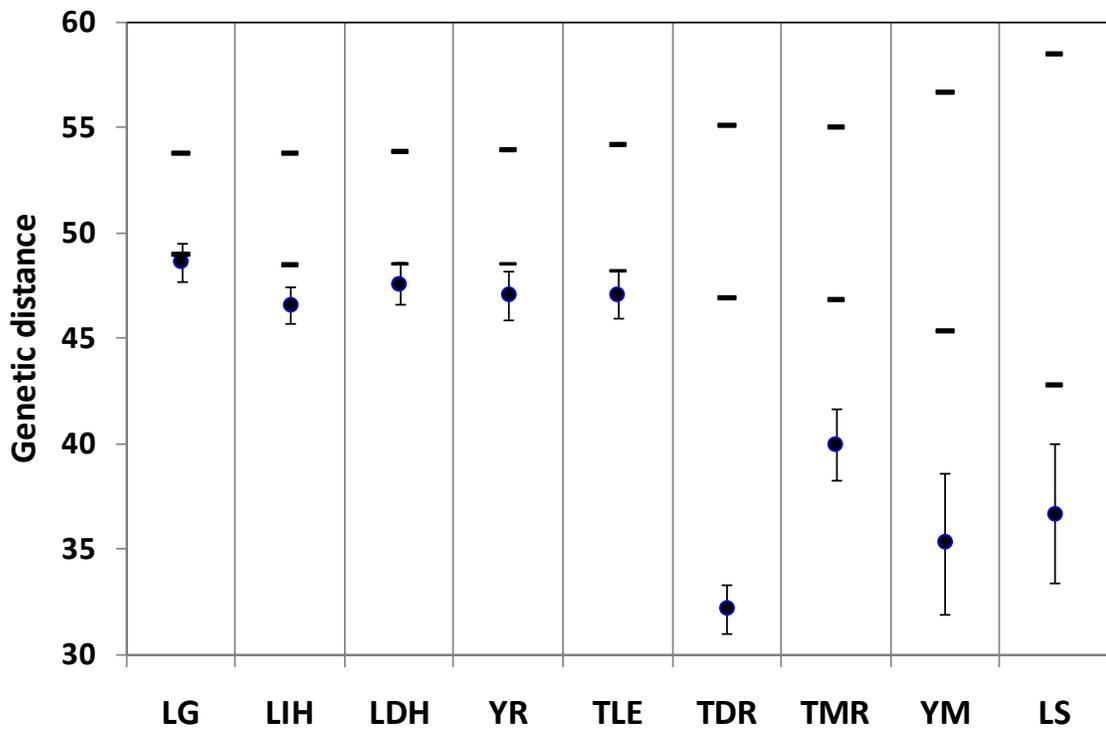


Figure 5 Mean within forest fragment pair-wise genetic distance values between individuals (bars with 95% CI). Grey lines indicate 95% CI around the random expectation.

Spatial Genetic Autocorrelation

Three of the sampled regions (LH, Y, LG) had positive spatial autocorrelation at short distance classes 5 – 25 m (Fig. 5). Within the LH region there was also significant positive spatial structure at 2 km, which is the distance between the LS, LDH, and LIH fragments indicating that some individuals between these fragments have similar genotypes. LG had comparatively weaker autocorrelation than either LH or Y, although 95% bootstrap values overlap at the shortest distance class for all three regions. The Tragadero region also had positive spatial autocorrelation out to 200 m before becoming significantly negative at 500 m. This pattern was due entirely to the fact that two of the Tragadero fragments were highly divergent from each other ($\Phi_{iPT} = 0.445$, Table 2). The positive spatial autocorrelation out to 200 m indicates that individuals within these two fragments were more similar to each other than between the two fragments where r becomes negative at 500 m. Separate analyses of each Tragadero fragment revealed no significant spatial autocorrelation at short distances (results not shown). I performed a combined spatial analysis for LH, LG, and Y regions since they had similar patterns (Fig. 6). This analysis revealed significant positive spatial structure between 5 and 50 meters with the correlogram crossing the y-axis at 200 meters.

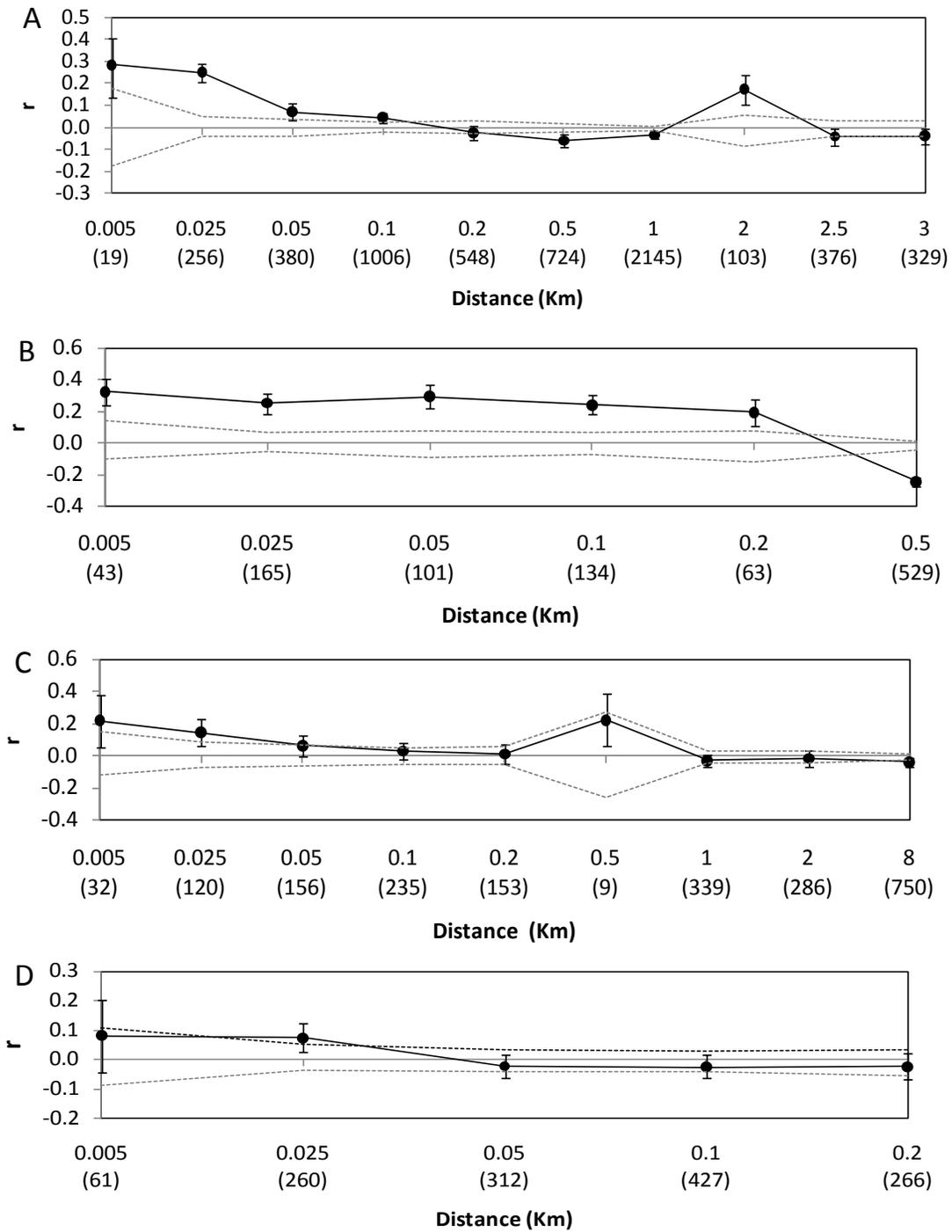


Figure 6 Genetic spatial autocorrelation of *Polylepis multijuga* individuals in the northern Andes (A) Laguna Huayabamba, (B) Tragadero, (C) Llihui, (D) Los Gevaras. Dashed lines are 95% confidence intervals around $r = 0$. Vertical lines are 95% bootstrapped confidence intervals around each calculated r value. Numbers in parentheses under the distance classes are the number of pair-wise comparisons in that class.

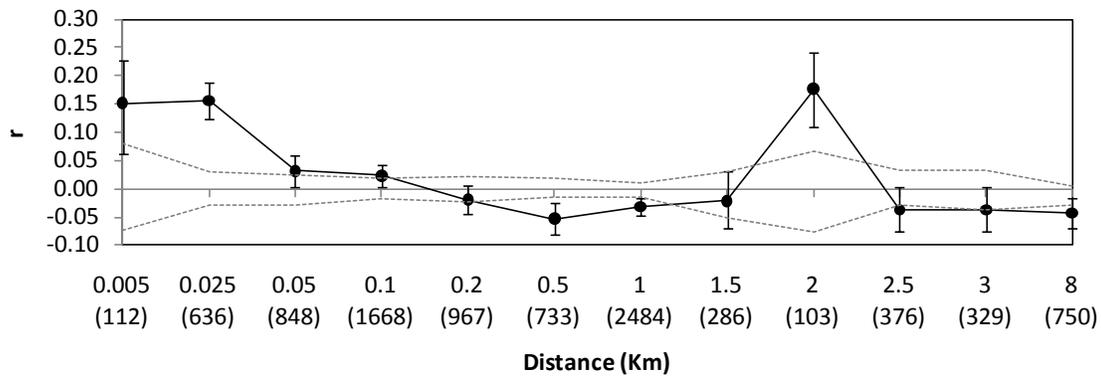


Figure 7 Combined genetic spatial autocorrelation of *Polylepis multijuga*. Dashed lines are 95% confidence intervals around $r = 0$. Vertical lines are 95% bootstrapped confidence intervals around each calculated r value. Numbers in parentheses under the distance classes are the number of pair-wise comparisons in that class.

Discussion

Comparative studies suggest endemic species with small geographic ranges have lower genetic diversity than more widespread closely related species (Karron 1987; Hamrick and Godt 1990; but see Nybom 2004). Average heterozygosity for *P. multijuga* (0.13) was lower than reported for other species with similar life histories (0.25 – 0.27) (Nybom 2004) and was also lower than reported in two studies of the endemic *P. australis* in central Argentina (0.27) (Julio et al. 2008; Seltsmann et al. 2009b) supporting the general assertion that species with small ranges also have low genetic diversity. The low genetic diversity found in some endemic species is predicted to increase their extinction risk due to inbreeding and reduced adaptability. Because endemics have small population sizes and limited distributions they may be less likely to adapt to new conditions created by climate change (León et al. 2006, Ford et al. 2009).

As expected for a wind-pollinated tree, *P. multijuga* had moderate differentiation across its range with only 14% of variation partitioned between fragments. This value was considerably higher; however, than that reported for two forest fragments of *P. australis* separated by 34 km ($F_{ST} = 0.02$). There is evidence for *P. australis* that pollen can travel more than 80 km and remain

viable for long time periods, thereby increasing the possibility of successful long-distance pollination (Seltmann et al. 2009a). *P. multijuga* pollen is also expected to travel long distances. Consistent with this expectation is a lack of isolation-by-distance between forest fragments. Nevertheless, pair-wise PhiTP values indicated that some of the sampled fragments are highly differentiated even though they are geographically close to each other. This differentiation may be caused by either a lack of gene flow between some fragments or it may be related to habitat variables that affect the survival and reproduction of *P. multijuga*. TDR was the most differentiated fragment and was located in a deep glacial tarn surrounded by moraines, while the other two most differentiated fragments (LS and YM) are small fragments located at higher elevations. In all three of these fragments (TDR, LS, YM), individuals are significantly more similar or more closely related to each other than individuals in other fragments. This suggests that these fragments may be more isolated from outside pollen flow than the other fragments and that some geographic features may enhance or impede pollen flow between fragments. For instance, mountain ridges can work as barriers that obstruct gene flow and ravines can function as corridors that enhance connectivity (Hu et al. 2010; Sork and Smouse 2006). Hu et al.'s (2010) research on *Fraxinus mandshurica*, a wind-pollinated tree, revealed differentiation between mountain and riparian populations but not between populations located within these habitat types suggesting there was lower gene flow between habitat types than within them. Marcora et al. (2008) found that higher elevations negatively affect seed and seedling survival. This could lead to higher levels of genetic drift in high elevation fragments and may also be responsible for the low genetic diversity in the LS and YM fragments ($H_e = 0.10$ and 0.098 respectively).

The positive spatial autocorrelation within fragments is probably related to short distance seed dispersal. Although *Polylepis* seeds are generally thought to be wind dispersed, Seltmann et al. (2009a) observed that a high proportion of seeds are dispersed by gravity alone.

A study of *P. australis* suggests that the majority of seeds travel no more than 6 m from the parent tree and seedlings were never found more than 10 m away (Torres et al. 2008). The relatively high correlation coefficients between trees at 5-10 m ($r \sim 0.20$) are consistent with these dispersal distances for seeds. There was variation between fragments in both the strength and presence of short distance spatial autocorrelation suggesting that seed dispersal distances also vary across this landscape. Some fragments did not exhibit short distance spatial autocorrelation such as those located in Tragadero or they only exhibited a weak pattern such as in Los Gevaras. Los Gevaras was the largest fragment and the lack of strong spatial autocorrelation may indicate either 1) the trees were older which tends to decrease spatial structure, or 2) seed dispersal in this fragment is enhanced relative to the smaller fragments. The lack of a pattern in the Tragadero fragments may simply be due to the fact that individuals in these fragments were genetically more similar to each other than in some of the other fragments and so there was not enough variability to detect a significant positive signal at short distances.

Conservation efforts for fragments of *P. multijuga* will need to take into consideration the surrounding landscape in order to predict whether fragments are likely to contain relatively high levels of genetic diversity and will be connected by gene flow to other fragments. *Polylepis* is vulnerable to selfing and biparental inbreeding (Seltmann et al. 2007, 2009 a, b) and so small, isolated fragments could potentially be augmented with individuals from other fragments to increase their diversity. Populations with high heterozygosity and low spatial autocorrelation such as LG could serve as sources of genetic variability for these isolated populations or seed sources for replanting efforts in areas where forest no longer occurs.

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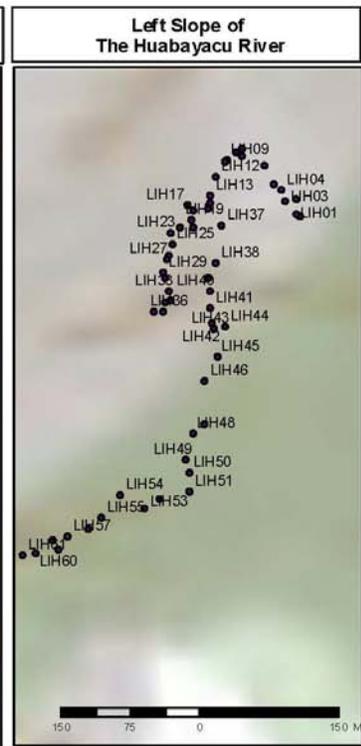
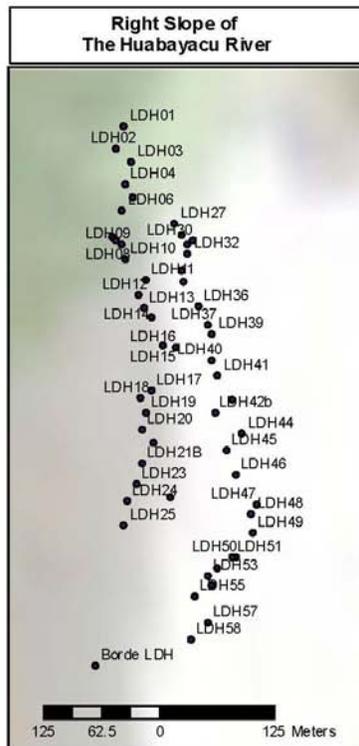
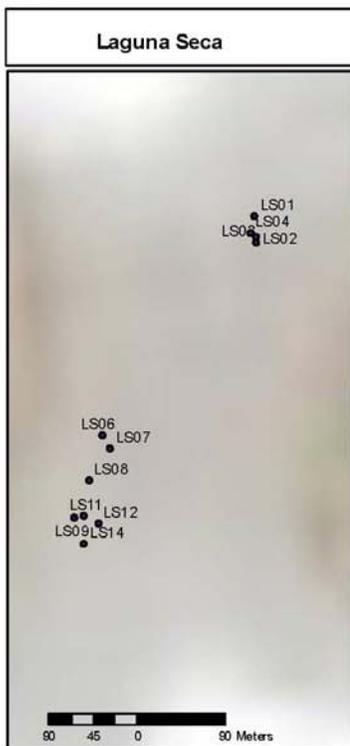
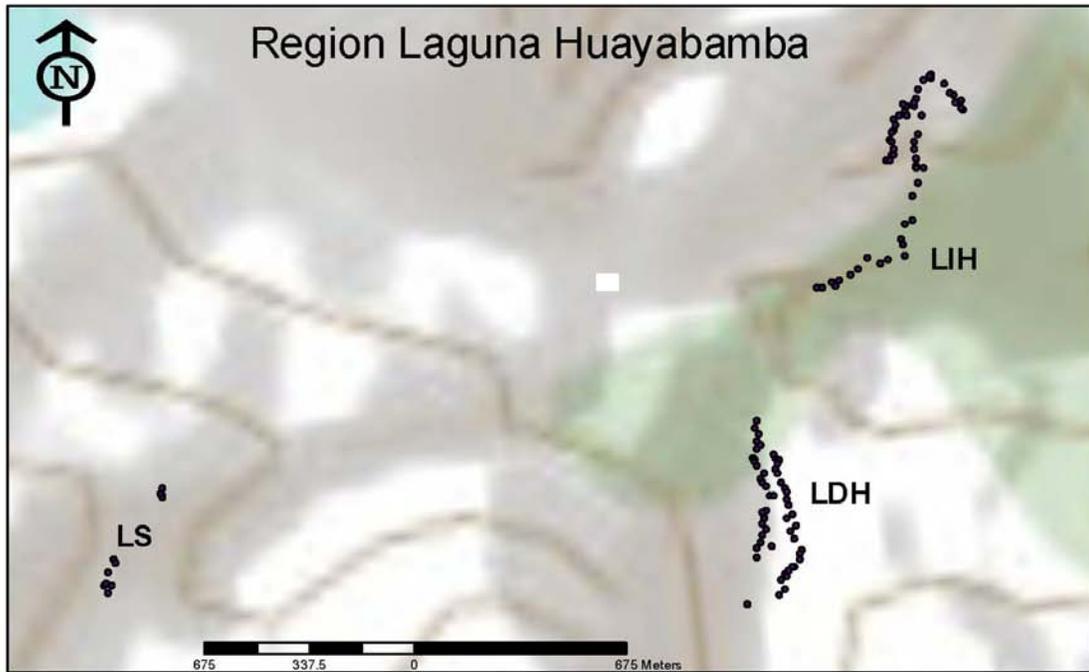
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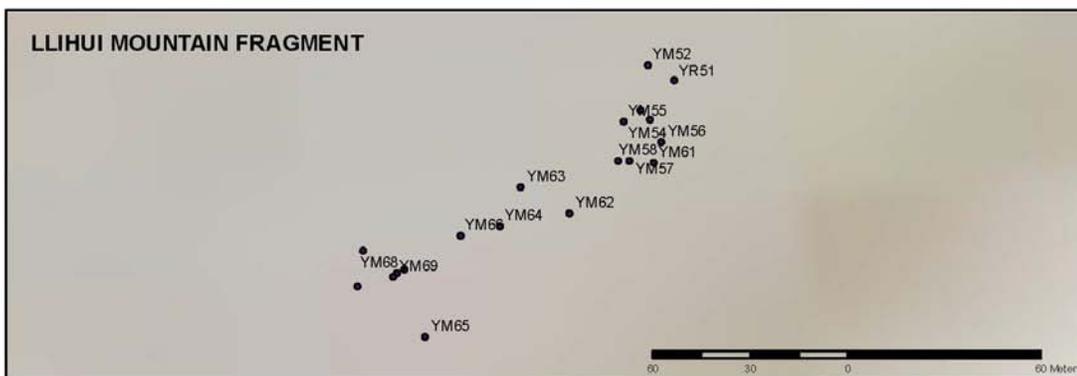
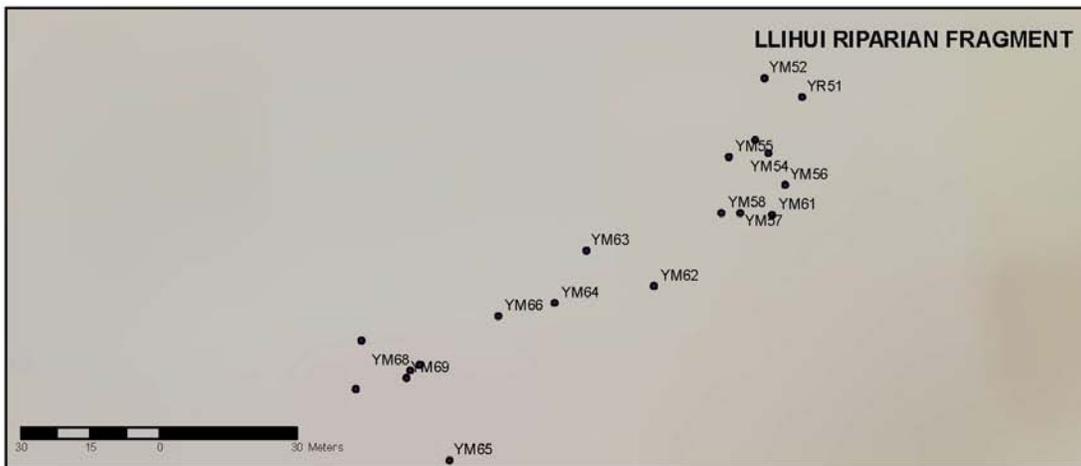
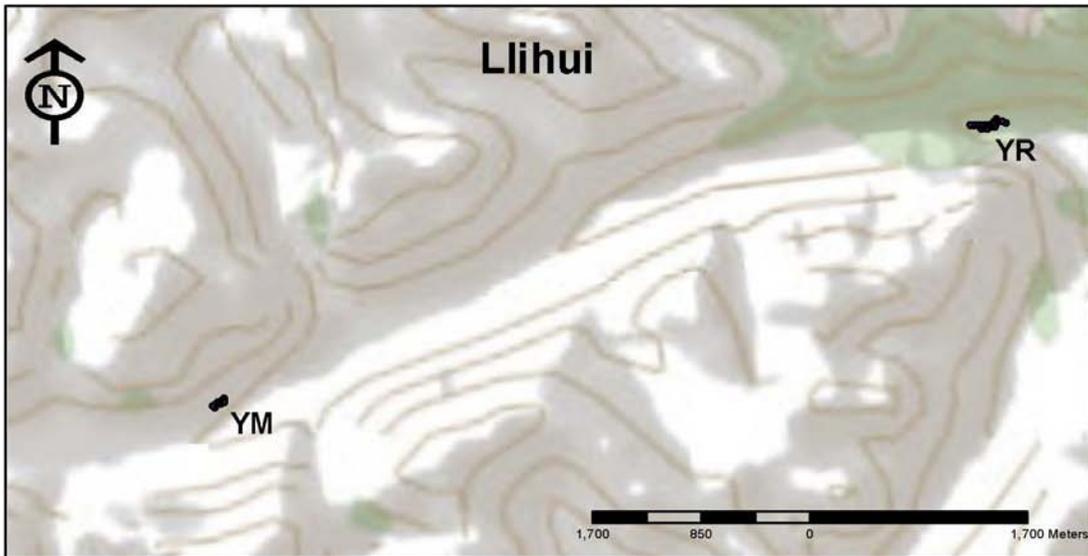
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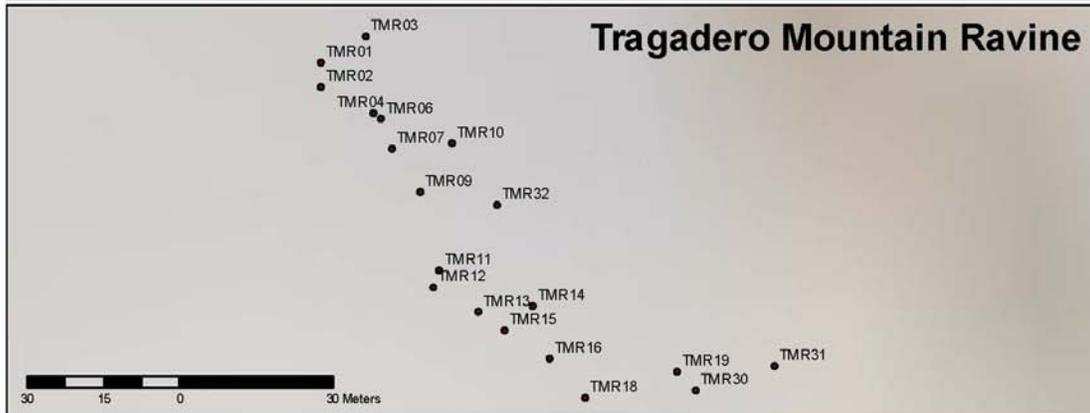
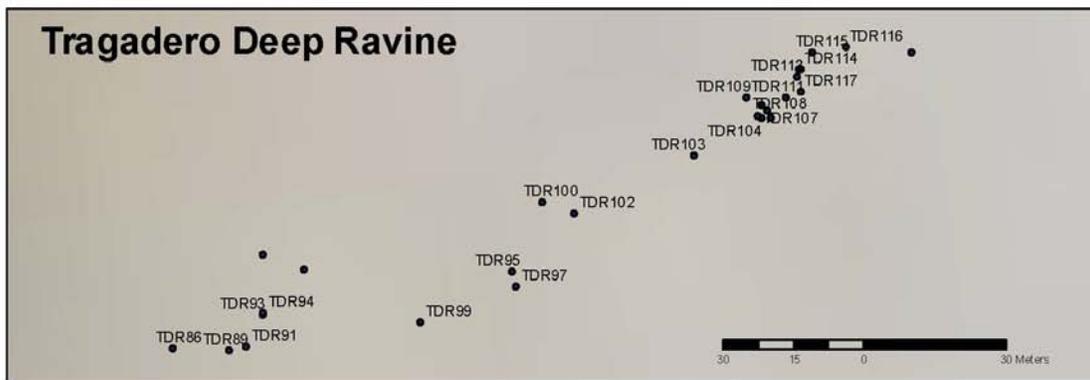
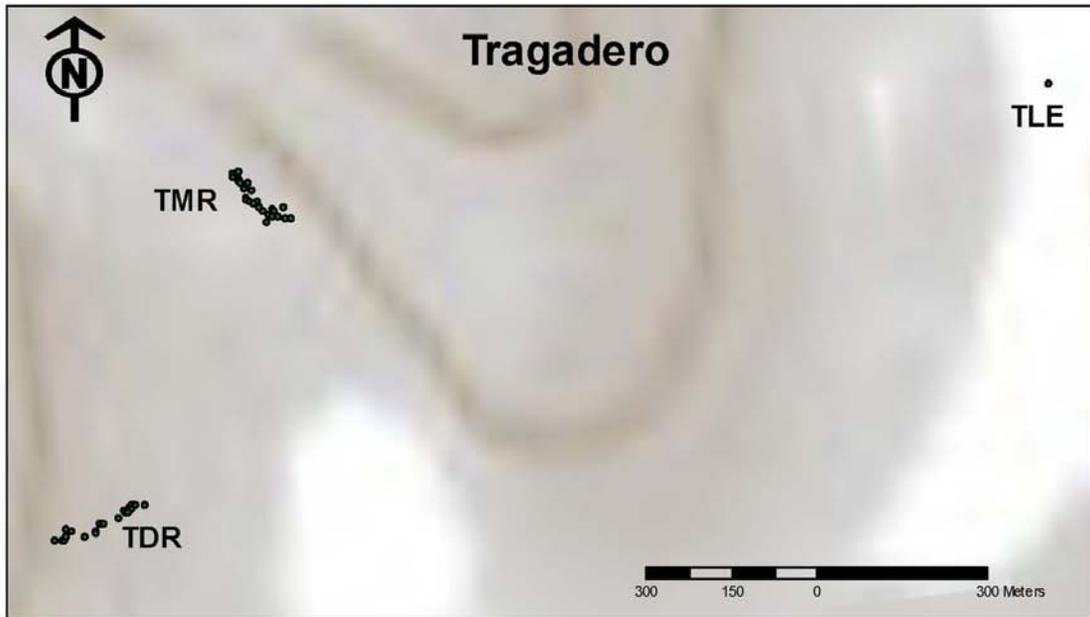
APPENDIX 1 Laguna Huayabamba - location of sampled fragments and individuals.



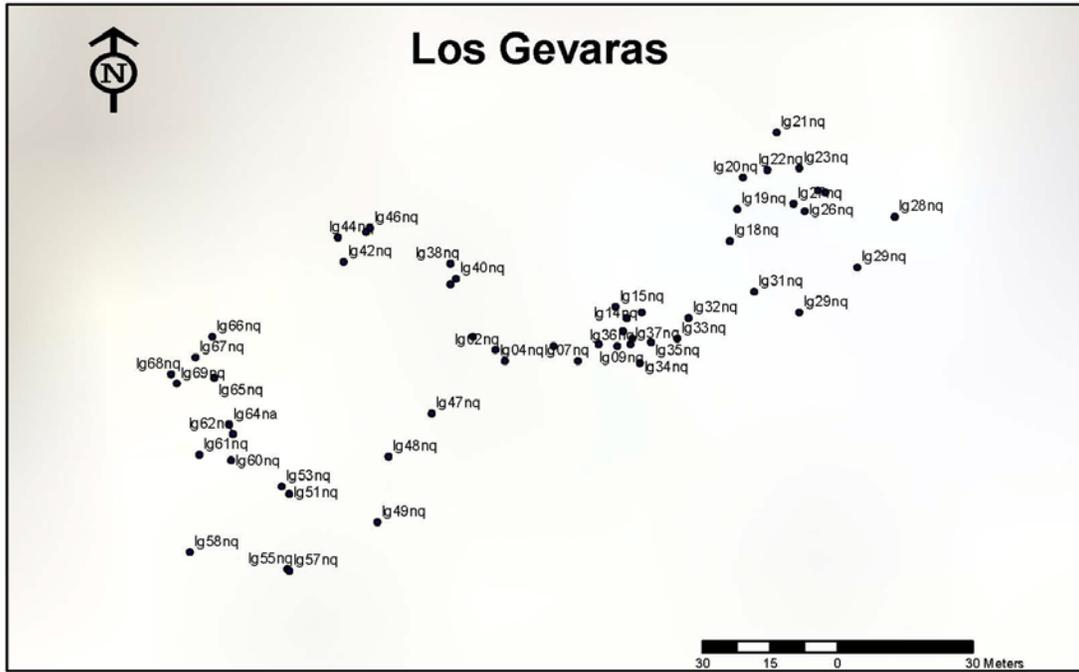
APPENDIX 2 Lihui map - location of sampled fragments and individuals.



APPENDIX 3 Tragadero map - location of sampled fragments and individuals.



APPENDIX 4 Los Gevaras map - location of sampled fragments and individuals.



VITA

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Organization for Tropical Studies
Madre de Dios – Peru, 2007

Experience Teaching Assistantship, Texas Christian University 2008-2010
Consultant for Amazon Conservation Association. Habitat Description Section – Plan De Sitio of Wayquecha Biological Station Manu National Park – Peru. May-July 2007
Consultant for the Frankfort Zoological Society Office – Peru. Conservation Status of Mahogany (*Swietenia macrophylla*). Jan – April 2007
Dr. John Terborgh’s Research Assistant. “Dynamics, forest growth, seeds dispersion and light measurement at Manu National Park and Manu Biosphere Reserve” May 2005 – May 2006
Resident Manager at Cocha Cashu Biological Station, Manu National Park – Peru. May 2005 – May 2006
Dr. Harald Beck’s Research Assistant. “Impacts of peccaries on plant communities in Los Amigos and Cocha Cashu” Los Amigos Research Station. Madre de Dios – Peru. June 2005
Conservation Status of Brazilian nuts (*Bertholletia excelsa*) forests in Peru. World Wildlife Fund Office – Peru March – April 2005

Dr. Nicole Gibson's Research Assistant. "Mating strategies and territoriality of male spider monkeys (*Ateles belzebuth chamek*) in Manu National Park, Peru. July – Oct. 2004

Consultant for SwissContact Office – Peru. Presence of Spectacle Bears (*Tremarctos ornatus*) at the left slope of the Apurimac Canyon. October 2004

Dr. John Bunce's Research Assistant. "Behavioral Genetics of Color Vision in a Wild Neotropical Monkey, *Callicebus brunneus*." Cocha Cashu Biological Station. Manu National Park – Peru. June – July 2003

Dr. Luis Paz Soldán's Research Assistant. "Anthropogenic impact over sea lions (*Otaria byronia*) and Humboldt penguins (*Spheniscus humboldti*) in the Isla de Asia colonies. NGO Pro Islas - Isla de Asia. Lima – Peru. Jan – March 2002

Margarita Ulenbrog's Research Assistant. Primary productivity of the Algarrobo (*Prosopis pallida*) in the Sechura Desert. May – June 1998

Volunteer Park Ranger. Lomas de Lachay Natural Reserve. Lima – Peru. Jan – Nov 2000

Awards

Environmental Science T.A. Award

ABSTRACT

GENETIC STRUCTURE OF FRAGMENTED *POLYLEPIS MULTIJUGA* PLIGE (ROSACEAE) FORESTS

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Department of Environmental Science

Texas Christian University

Thesis Advisor: Dean Williams, Assistant Professor of Biology

Polylepis multijuga is a threatened, endemic tree species in the northern Andes of Peru. *P. multijuga* is wind-pollinated which could allow for gene flow among fragments that would counteract drift and loss of genetic diversity. I collected samples from 371 trees in nine forest fragments and genotyped them at 316 AFLP loci. Genetic diversity ($H = 0.131$) was low compared to other species and genetic differentiation across all fragments was moderate ($\Phi_{iPT} = 0.14$). Short distance (5-50m) genetic spatial autocorrelation within most fragments indicated short distance seed dispersal. Pair-wise genetic distance estimates between fragments (range: 0.036 – 0.487) were not related to geographic distance and instead appeared to be related to habitat. The three most differentiated fragments, which also had the lowest genetic diversity values, were two small, high altitude fragments and a fragment located in a deep ravine suggesting that pollen flow does not effectively connect all fragments in this landscape.