CONSERVATION OF THE TROPICAL HIGH MOUNTAIN TREE GENUS *Polylepis* IN BOLIVIA

A COMBINED ECOLOGICAL AND GENETIC APPROACH

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PREFACE

I started to work on *Polylepis* woodlands after deciding the subject topic of my Licenciate in Biology thesis around year 2000. At that time, Milton Fernández offered me the opportunity to do science in order to conserve a native threatened species, *P. besseri*. It had a vulnerable conservation status according to IUCN and the results obtained would help their conservation. These reasons were enough to decide to estimate the radial growth rate of these trees. It was a challenging task because I had to learn something completely new for me, dendrochronology. Two years after I finished my Licenciate thesis I received an invitation to participate in a course taught by Javier Simonetti, Audrey Grez and Ramiro Bustamente on habitat fragmentation organized by the Centro de Biodiversidad y Genética (CBG) de la Universidad Mayor de San Simón (UMSS). I was hired by CBG to study the effects of the exotic species *Eucalyptus globulus* and *Pinus radiata* on the regeneration of *Polylepis subtusalbida* a few months after that course. This study was part of a larger project: “Conservación de los bosques de Polylepis en el Parque Nacional Tunari” (COBAT) funded by VLIR-IUC (Belgium) having Erik Matthysen as Belgian supervisor. Part of the results was published thanks to the collaboration with Yvonne Y. Martinez, Luis F. Aguirre, Ramiro O. Bustamante, and Melicio M. Siles and they are part of this PhD thesis (Chapter 5). During my work on CBG, Jennifer Cahill proposed a project to give biological and genetic basis for the future reforestation with *P. besseri* in Cochabamba that was funded by ASDI (Sweden) and in which I was lucky to participate with the collaboration of Freddy Navarro, Yvonne Y. Martinez and others, including Susana Arrázola as CBG Director. It was then, that I received a letter with information about scholarships between KU Leuven and UMSS. It was the start of this PhD journey.

Before I came to Leuven I had to choose a supervisor and to present them a PhD project. I selected two, the best I could select: Olivier Honnay and Martin Hermy. I introduce them the study system and we developed a project to help the conservation of *Polylepis* woodlands in Bolivia, and you are reading the result. Once in Leuven I shared the room with Tine Meekers and

Tobias Ceulemans, Filip Vandelook and Maarten Vanderstukken. Thank all of you for helping me make this dream come true.

I am very grateful with the help of all these people who helped me in this adventure of becoming a scientist. Specially thanks to my supervisors Olivier Honnay and Martin Hermy. Thank you for your support during the PhD formation and during one of the most difficult times in my life. Erik Matthysen, thank you for being the link between Belgium and Bolivia at that time. I am also grateful to the members of my Examination Committee Ellen Decaestecker, Bart Muys, Koenraad Muylaert, Filip Vandelook, and Erik Matthysen, who taught me to exploit myself in the benefit of others. To those who collaborated with me as co-authors, namely Yvonne Y. Martinez, Luis F. Aguirre, Milton Fernández, Sharon Stanton, Jon Fjeldså, Filip Vandelook, Peter Breyne, Katrien Vandepitte, Jennifer R.A. Cahill, Ramiro O. Bustamante, and Melicio M. Siles, and the anonymous reviewers who improved the manuscripts, thank you. I could not do all this job without you. Also for those who collaborated in getting the data and/or analyzing them (A todos aquellos que colaboraron en la colecta y/o análisis de datos): Staff of the Parque Nacional Tunari, Luzmila Flores, Luis Cerezo, Justiniano Arispe, Communities Pajcha, Pintumayu, Thola P’ujru, Sacha Loma, Cuturi, Saúl Altamirano, Frederic Lens, Staff CBG, Gonzalo Navarro, Christa De Troeyer, Geert Verbeke, Modesto Zárate, José Antonio Balderrama, Teresa Tarifa, Lidia Meneses, Gabriela Uriona, Natalia Zambrana, Kasper Van Acker, Kent J. Bradford, Hans Jacquemyn, Luis Alvarez, Henk Hilhorst, Esmeralda Ballesteros, Marta Morales, Sabrina Neyrinck, Nancy Van Liefferinge, David Halfmaerten, Leen Verschaeve, and An Van Breusegem, thank you for showing me that love for science is more than love for oneself, selfishness.

A mis padres Edith León y Alfredo Gareca, muchas gracias por el apoyo brindado. A la mujer que Dios puso en mi vida Yvonne Y. Martinez y al hijo que Él nos regaló Isaac A. Gareca, sin ustedes esta aventura no hubiera sido posible.

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SAMENVATTING

"*Conservation Biology*" is een discipline die geen correcte Nederlandse vertaling heeft en kan worden gedefinieerd als de multidisciplinaire wetenschap die tot doel heeft het verlies aan biodiversiteit op aarde te stoppen, en lokaal verlies van biodiversiteit te herstellen. *Conservation Biology* beschikt over het conceptuele kader en de nodige instrumenten om de bedreigingen voor de *Polylepis*wouden in de hoge Zuid-Amerikaanse Andes te kwantificeren en om maatregelen voor behoud en herstel te formuleren. De belangrijkste concrete bedreigingen van de *Polylepis*wouden zijn branden, overexploitatie, overbegrazing, herbebossing met exotische boomsoorten, habitatfragmentatie en de opwarming van het klimaat. Momenteel ontbreekt het aan informatie omtrent de gevolgen van habitatfragmentatie, de aanplant van exoten en de opwarming van het klimaat op de *Polylepis*wouden in de hoge Boliviaanse Andes, nochtans een belangrijk centrum van *Polylepis*-endemisme in Zuid-Amerika. De belangrijkste doelstelling van dit werk was dan ook de studie van de effecten van de drie vermelde bedreigingen van *Polylepis*wouden in Bolivië. Daarbij werd een benadering gehanteerd die veld- en laboratoriumexperimenten combineert met observationele studies. Een inleidende overzichtsstudie van de biodiversiteit in Boliviaanse *Polylepis*wouden leverde, naast 13 soorten van het *Polylepis*genus, een soortenlijst op met 780 soorten, inclusief vogels, reptielen, planten en vlinders. Tien van de 13 *Polylepis*soorten, 7 plantensoorten, 14 vogelsoorten en 4 zoogdiersoorten werden door de IUCN gecategoriseerd als bedreigd of bijna bedreigd. Macro-ecologische patronen gegenereerd op basis van literatuurgegevens suggereren nog dat de planten- en vogelsoortenrijkdom van Polylepiswouden toeneemt met een toename van de jaarlijkse neerslag en van de lengte van het groeiseizoen. Wat betreft de effecten van de opwarming van het klimaat op kieming van zaden van *P. besseri* kon op basis van hydrothermale tijdsmodellen worden vastgesteld dat een toename van 0.3 graden Celsius van de huidige minimum omgevingstemperatuur, *P. besseri* kan vrijstellen van de limiet die momenteel wordt opgelegd door de basistemperatuur van de soort (3 graden Celsius).

Indien de omgevingstemperatuur verder toeneemt zal de resulterende zaadkieming beïnvloed worden door zowel de stijgende temperatuur als de resulterende gereduceerde waterpotentiaal. Pas wanneer de maximale omgevingstemperatuur boven de 21.7 graden Celsius zal uitstijgen zal de kieming van *Polylepis*zaden weer beginnen afnemen. Zaden van *P. besseri* afkomstig van de grotere hoogtes in de Andes zouden onder deze omstandigheden evenwel onaangepast aan hun omgeving worden, gegeven hun thuisvoordeel in koude omgevingen. Indien deze resultaten bevestigd worden op basis van multi-jaar studies dan dient de translocatie van zaden van lage naar grote hoogteligging te worden overwogen. Wat betreft de regeneratie van *Polylepis*wouden in een matrix van exotische boomsoorten kon worden vastgesteld dat zaailingen van de tweede studiesoort, *P. subtusalbida*, kunnen co-existeren met exotische boomsoorten zoals *Pinus radiata* en *Eucalyptus globulus*. Deze co-existentie gebeurde evenwel bij lage plantdensiteiten en relatief lage kroonsluiting. De effecten op de mortaliteit bleken sterk afhankelijk te zijn van de lokale omstandigheden, wat suggereert dat een sterk lokaal gericht herstelbeleid noodzakelijk is opdat dit herstel duurzaam zou zijn. Inzake de effecten van omgevingstemperatuur op de groei van *Polylepis besseri,* leverde ook de dendrochronologische analyses verschillen op tussen de diverse sites. Of er een positieve respons is van de groei, volgend op een temperatuursstijging net voor het groeiseizoen, bleek sterk afhankelijk van de lokaal heersende klimatologische omstandigheden. De studie van de populatiegenetica van *P. besseri*, in combinatie met een literatuurstudie van alle beschikbare populatiegenetische data voor *polylepis*soorten, leverde als conclusie op dat de belangrijkste habitatfragmentatie-processen zich relatief recent dienen te hebben afgespeeld aangezien habitatfragmentatie een nog relatief beperkt effect blijkt te hebben op de verdeling van de genetische variatie tussen en binnen habitatfragmenten. Mogelijk viel de habitatfragmentatie samen met de verovering van Zuid-Amerika door Spaanse troepen vanaf 1600. Dit impliceert dat het verspreidingsgebied van *Polylepis* veel omvangrijker is dan de huidige verspreiding doet vermoeden en dit opent perspectieven voor herbebossingsinitiatieven.

SUMMARY

Conservation biology, defined by its goal—to halt or repair the undeniable, massive damage that is being done to ecosystems, species, and the relationships of humans to the environment, can help to understand how native species respond to the main threats to biodiversity. The tropical Andes is one of the most important mountainous hotspots of biodiversity, and the ecosystems above the forest line contain one of the most threatened ecosystems in South America, the *Polylepis* woodlands. The main threats to these ecosystems are fire, overcutting, overgrazing with cattle and sheep, and reforestation with exotic tree species, as well as habitat fragmentation, and global warming effects. In Bolivia, core area for the genus' diversity, there is little or no information about the effects of exotic species, fragmentation and climate change on *Polylepis* species. Our research aims to describing the species diversity inhabiting *Polylepis* woodlands in Bolivia, and studying how *Polylepis* trees and populations may respond to three major threats: global warming, the introduction of exotic species and habitat fragmentation. We used literature reviews, observational studies, and field and laboratory experiments to understand how the regeneration, growth and population genetics of the native trees *Polylepis* (*P. besseri* and *P. subtusalbida*) respond to these threats. The Bolivian high Andes holds 13 species and three subspecies of the genus *Polylepis*, which including plants, birds, mammals, butterflies and reptiles, at least contain 780 species. Ten of the 13 *Polylepis* species, as well as 7 other plants, 14 birds and 4 mammals were categorized as threatened or near threatened according to IUCN criteria, showing it is time to act to conserve these species. Herbs and shrubs were most common plants found in the *Polylepis* woodlands in Bolivia, whereas insectivorous birds, mammals, and reptiles were the most dominant guilds for animals. However, we found only one study dealing with insects in the *Polylepis* woodlands. Increasing the length of the growth season and precipitation, both increased plant and bird species richness. Regarding the effect of global warming on *P. besseri*'s regeneration, and based on the hydrothermal time modelling of germination, we found that an increase of 0.3°C of the current minimum field temperature may release *P. besseri* from the limit imposed by its base temperature (3°C). As temperatures rise, germination will be the result of the balance between increased temperatures and reduced water potential. Finally, when maximum temperature surpasses the optimum temperature for *P. besseri* (21.7°C), germination will start to decrease. *P. besseri* seeds from high elevations might become maladapted to warm conditions, given their home-site advantage for cold environments. If this pattern of local adaptation is supported through different years, then the translocation of individuals from low to high elevation has to be considered. Regarding *Polylepis*' regeneration with exotic trees, we found that *P. subtusalbida*'s seedlings may coexist with the exotic trees *Pinus radiata* and *Eucalyptus globulus.* Likely, this coexistence occurs under low plant densities and low canopy cover. Although it depended on the locations, exotic species increased the frequency of the lateral growth form in *P. subtusalbida*, which in turn have the lowest survival. Thus, any measure for sustainable management of these forests should be contingent to specific locations. As for what concerns the effects of global warming on *Polylepis* growth, we found that an increase of temperature before the growth season (October) would increase *P. besseri*'s growth at Uypa fragment but not at Chutu Senega because trees from both fragments respond in different ways to regional climate. Our literature survey on the effects of habitat fragmentation on population genetics of *Polylepis* woodlands showed that offspring lost genetic diversity and increased its differentiation compared to the adult generation. These results show fragmentation effects are only becoming apparent, and suggest the main fragmentation event occurred *c*. 400yr ago. Manual introduction of pollen or seed from other fragments can be an efficient conservation strategy that needs to be initially evaluated through field experiments. Finally, we present unsolved problems and suggestions for further research, which include and are not limited to the modelling of germination below the base temperature for *P. besseri*, the confirmation of the local adaptation pattern to high elevations of the germination, the identification of the limiting factors for grassland colonization, and field surveys through the country to complete accounts of species diversity of *Polylepis* woodlands.

LIST OF ABBREVIATIONS

BP	before present
mya	million years ago
ENSO	El Niño Southern Oscillation
LGS	length of the growth season
PCA	principal component analysis
PET	potential evapotranspiration
PNT	Parque Nacional Tunari
SST	Sea surface temperature

LIST OF SYMBOLS

c	relative change in individual size
F_{IS}	Inbreeding coefficient
h_0	seedling height in the 2004 sampling period
h_1	seedling height in the 2005 sampling period
H_e	expected heterozygosity
k_T	slope of the relationship between $\psi_{b(g)}$ and temperature in the supra-optimal range of temperatures
MV	molecular variance
PPL	percentage of polymorphic loci
T	temperature in the experiment
T_b	base temperature for germination
$T_{c(50)}$	mean ceiling temperature
t_g	time to germination of the g fraction
T_o	optimum temperature for germination
θ_C	thermal time constant at supra-optimal temperatures
θ_H	hydrotime constant
θ_{HT}	hydrothermal time constant
$\theta_{T(50)}$	mean thermal time
σ_{Tc}	standard deviation of the ceiling temperatures
$\sigma_{\theta T}$	standard deviation of thermal time
$\sigma_{\psi b}$	standard deviation of the base water potential
Φ_{SC}	genetic differentiation between woodlands within regions
Φ_{ST}	genetic differentiation among *P. besseri* woodlands
ψ	water potential of the experiment
$\psi_{b(50)}$	mean base water potential

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The population genetics of fragmented *Polylepis* woodlands

Adapted from Gareca, E.E., P. Breyne, K. Vandepitte, J.R.A. Cahill, M. Fernández, & O. Honnay. Genetic diversity of the Andean *Polylepis* woodlands and inferences regarding their fragmentation history. *Conservation Genetics* (submitted).

General discussion and conclusions

CHAPTER 1

Introduction

Mountain forests as biodiversity hot spots

Conservation biology is defined by its goal—to halt or repair the undeniable, massive damage that is being done to ecosystems, species, and the relationships of humans to the environment (Ehrenfeld 1992). Increasing human population size and per capita consumption have generated the sixth mass extinction (Sodhi & Ehrlich 2010; Barnosky *et al.* 2011). The main causes identified include habitat destruction and fragmentation, overharvesting, invasive species, climate change, and fire (Sodhi & Ehrlich 2010). To understand how native species respond to these threats is of primary importance. Because it is predicted to be difficult to conserve all species on Earth, given the available resources, hotspots of biodiversity that contain exceptional concentrations of unique species (endemics) and that experience exceptional local loss of habitat have been identified all over the globe (Myers *et al.* 2000). Mountain systems such as those in Mesoamerica, the Caucasus, the Eastern Arc mountains of Tanzania and Kenia, and South Central China belong to these hotspots of biodiversity (Myers *et al.* 2000). Mountains cover 24% of the global land area, and mountain forests represent 28% of the world forests (Kapos *et al.* 2000). Mountain forests offer a variety of ecological services to humans (Douglas 1999; Sidle *et al.* 2006). They are key sources of wood and non-wood products for human use (Smiet 1992; Moraes *et al.* 1995; Molles Jr. 2002), they mitigate local climate, are an important source of water for domestic, agricultural, and industrial consumption (Beniston 2003; Buytaert *et al.* 2006a), reduce soil erosion, improve soil nutrient status, and they are important biodiversity hotspots (Burgess *et al.* 2007; Menegon *et al.* 2008). One of the globally most important mountainous hotspots of biodiversity is the tropical Andes.

The tropical Andes are distributed from Venezuela in the north (11°N) to the border between Chile and Argentina in the south (24°-27°S), and their altitude range from 600-800m to 6000m a.s.l. (Emck *et al.* 2006; Josse *et al.* 2011). The treeline, which occurs between 3800-4500m near the equator, and the snow line (occurring between 4500-5000m a.s.l. near the equator), both decrease towards the southern limit of the region (Josse *et al.* 2009; Josse *et al.* 2011). Because the environmental conditions change along the altitudinal gradient, several life zones may be found on a single mountain side (Molles Jr. 2002; Ghalambor *et al.* 2006). The ecosystems above the forest line in the tropical Andes in South America are known by their most distinctive local plant formations, generally defined as *Páramo* in the north, and *Puna* in the central Andes (Vuilleumier & Monasterio 1986). These landscapes are patchy and consist of a mosaic of low grass and scrub with occasional oases of more dense vegetation mainly dominated by monospecific stands of one arborescent genus, *Polylepis*.

The genus *Polylepis* & *Polylepis* woodlands

The genus *Polylepis* (Rosaceae) consists of shrubs and trees with usually twisted trunks, which can reach a height of 1-27m and a diameter of 2m (Simpson 1979; Fjeldså & Kessler 1996; Fig. 1.1b). They are evergreen with small and sparsely to densely packed leaves and usually contain large amounts of small dead branches in the lower part of the canopy. The bark is thick and rough with even 150 layers which are easy to peel of (Fjeldså & Kessler 1996). The flowers of *Polylepis* are inconspicuous (about 4-10mm in diameter), apetalous, protogynous, and arranged in racemes (Fig. 1.1c). Most species are probably wind pollinated, and self pollination through geitonogamy may occur (Simpson 1979; Simpson 1986). The fruits are small (as the flowers), indehiscent, one seeded achenes, composed of the floral cup fused to the ovary. The fruit surface of different species has ridges, knobs, spines, or wings, also suggesting animal dispersal (Simpson 1986), although wind dispersal may predominate in some species (*e.g. P. australis*, Simpson 1979).

At the species level, the delimitation of taxa in the genus is difficult due to extensive morphological variability within populations, limited variability between many of the recognized species, and extensive hybridization (Kessler & Schmidt-Lebuhn 2006). The number of species described within the genus ranges from 15 to 33 (Simpson 1979). A recent study recognizing morphologically, biogeographically and ecologically distinct populations at species rank, suggested *c*. 26 *Polylepis* species (Kessler & Schmidt-Lebuhn 2006). The number of *Polylepis* species per country is 1 in Venezuela, 3 in Colombia (1 endemic), 7 in Ecuador (2 endemics), 14 in Peru (3 endemics), 13 in Bolivia (4 endemics), 2 in Chile, and 4 in Argentina (1 endemic).

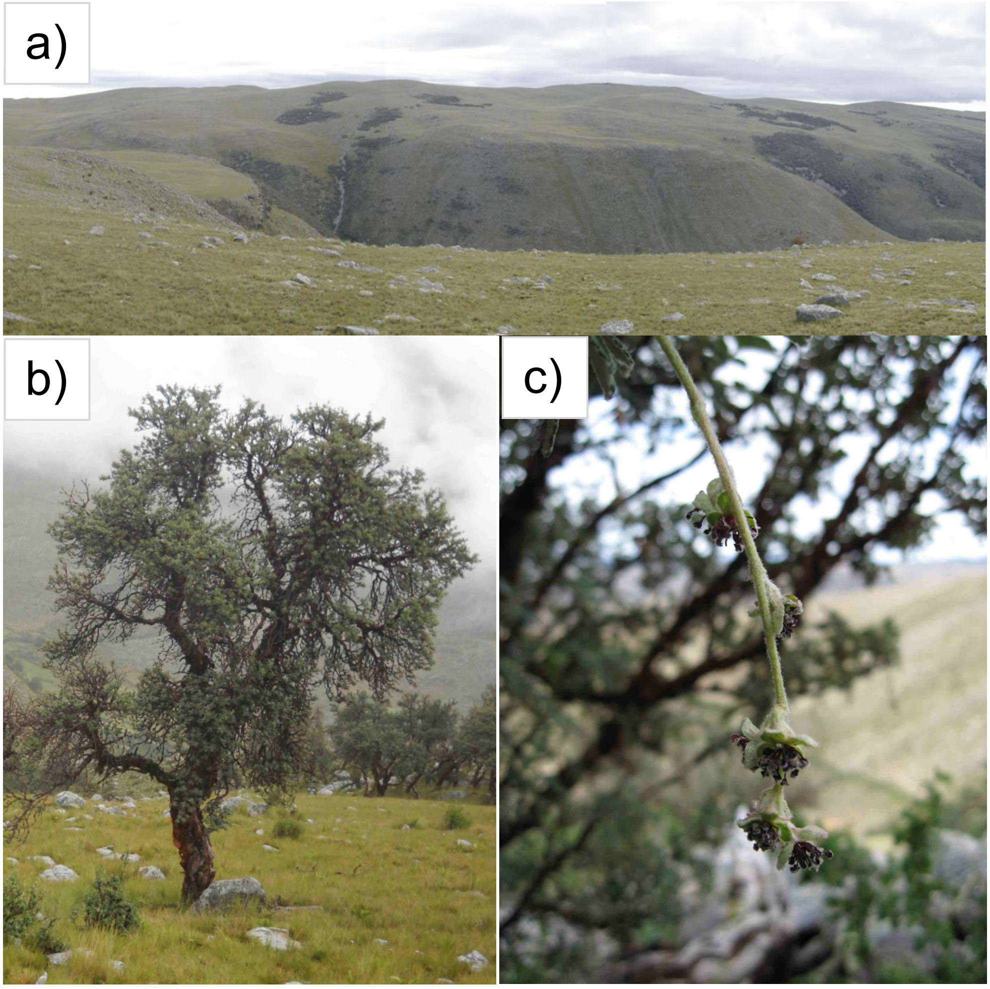

Fig. 1.1 *Polylepis besseri* Hieron. woodlands (a), tree (b), and inflorescence (c).

Polylepis woodlands (*e.g.* Fig. 1.a) are distributed along the Andes from Venezuela (9°N) to Argentina (32°S), and reach elevations as high as 5000-5200m (Simpson 1979; Navarro *et al.* 2005). Although at lower altitude *Polylepis* woodlands can be more continuous, most of them are isolated patches in landscapes dominated by grasslands, and they are often found on rocky slopes or along streams (Simpson 1979; Fjeldså & Kessler 1996). In the arid highlands, and in areas with little regeneration and strong impact of grazing and fires, the *Polylepis* woodlands are open and bushy, and the undergrowth differs little from the surrounding bunchgrass vegetation; unlike in mature and continuous forests in more humid zones, which present clear tree, shrub and herb layers, and a rich associated flora of herbs and vines, absent in the surrounding vegetation (Gareca *et al.* 2010b, see Chapter 2).

There is a long-standing debate on whether the current occurrence of *Polylepis* woodlands as relatively small and isolated fragments in a grassland matrix is a natural phenomenon (‘the natural fragmentation hypothesis’), or whether its originally more continuous natural distribution was largely modified by human use (‘the anthropogenic fragmentation hypothesis’) (Kessler 2002). At the beginning of the 20th century, early explorers have suggested that the scattered occurrence of *Polylepis* woodlands was governed by microclimatic variation. This was because many *Polylepis* woodlands occur at specific sites such as ravines, boulder slopes, rock faces, where particular microclimatic conditions (higher temperatures and humidity) provide a niche for the species (reviewed in Kessler 2002). In 1958, Hans Ellenberg has challenged this view and has hypothesized that a long history of fires inflicted by humans, livestock grazing and timber extraction has eventually resulted in the loss and fragmentation of most of the original *Polylepis* woodlands which are now confined to inaccessible rocky slopes and outcrops (Kessler 2002). The first indication of human manipulation of the high Andean environment occurred 7000-3000yr BP (Baied & Wheeler 1993; Kessler & Driesch 1993). The losses of *Polylepis* woodlands have been suggested to have occurred 5000-3000 BP, and after a period of forest recovery at 1000 BP, forest degradation and fragmentation increased again following the beginning of the Spanish conquest (AD 1533) (Kessler & Driesch 1993).

During the 1990ies, biogeographical and ecological evidence accumulated, supporting the anthropogenic fragmentation hypothesis (Kessler 1995; Fjeldså & Kessler 1996). In fact, it has been proposed that *Polylepis* occupies only 11% of the 51,000 km^2 of their potential area in Bolivia (Kessler 1995). Recently, however, Gosling *et al.* (2009) have suggested, based on pollen occurrence, that *Polylepis* has never formed natural continuous woodlands. They proposed that fire, a natural component of Andean ecosystems, could provide at least a partial explanation for the fragmented nature of *Polylepis* woodlands through the last 370,000yr in the high central Andes.

Polylepis woodlands are culturally important because local people use the kewiña or queñua (*Polylepis*) woodlands as a source of firewood, coal and wood, as well as sleeping places for cattle or as croplands (Yallico 1992; Lazcano & Espinoza 2001; Espinoza 2001). The associated plants to the *Polylepis* woodlands are used as source for food, medicines or for religious ceremonies, and they also contain forage species for cattle grazing (Kessler 2006). *Polylepis* woodlands, as other mountain forests, are also ecologically important because of the ecosystem services they provide, such as local increase in precipitation and water retention, avoidance of soil erosion and accumulation of nutrients in the soil (Fjeldså & Kessler 1996; Fernández *et al.* 2001). They also contain a high biodiversity compared to the surrounding grassland (Fjeldså & Kessler 1996; Kessler 2006; Chapter 2).

Threats to *Polylepis* woodlands

It has been stated that ecologically unsound land use methods are the main threat to *Polylepis* woodlands (Fjeldså & Kessler 1996); these include uncontrolled use of fire, overgrazing, overcutting, strong preference of people for the use of cattle and sheep, and reforestation with exotic tree species of the genus *Pinus* (Pinaceae) and *Eucalyptus* (Myrtaceae). So far, research has been focused on the effects of fire (Kessler & Driesch 1993; Renison *et al.* 2002; Cierjacks *et al.* 2008b; Urrego *et al.* 2011; Williams *et al.* 2011), cattle grazing and trampling (Teich *et al.* 2005; Cierjacks *et al.* 2008a; Zimmermann *et al.* 2009), overcutting because of increased accessibility due to road building and mining (Purcell & Brelsford 2004; Toivonen *et al.* 2011), fragmentation (Seltmann *et al.* 2007b; Hensen *et al.* 2012; Quinteros-Casaverde *et al.* 2012) and climate change (Marcora *et al.* 2008).

It has been reported that fire reduced reproduction by seed in *P. besseri*, *P. crista-galli*, *P. racemosa*, *P. incana* and *P. australis* (Kessler & Driesch 1993; Renison *et al.* 2002; Cierjacks *et al.* 2008b). Seedling population size decreased after fire in *P. incana,* despite the higher seedling survival in burned places (Cierjacks *et al.* 2008b). Increasing fire intensity reduced the relative frequency of young *P. besseri, P. crista-galli*, and *P. racemosa* trees with diameters <1cm (Kessler & Driesch 1993; Kessler 1995). Fire also reduced the survival of *P. australis* and *P. incana* trees (Renison *et al.* 2002; Cierjacks *et al.* 2008b). Furthermore, fire reduced the growth in height of *P. australis* trees, which showed the maximum biomass production at intermediate percentages of burned trees (Renison *et al.* 2002). This increase in biomass production, next to the decrease in growth in height, has likely caused the scrub-like physiognomy of *P. australis* trees (Renison *et al.* 2002), typical also for other *Polylepis* species such as *P. tomentella* and *P. pepei* (Kessler & Driesch 1993). The effects of fire on *Polylepis* were also studied on a longer time scale by the use of fossil pollen and charcoal, found in lake sediments. Evidence suggests that fire was a natural feature of high Andean ecosystems during the last 370,000yr and its persistent presence may be the mechanism responsible for the fragmented nature of *Polylepis* woodlands through the whole period (Gosling *et al.* 2009).

Since most of the current use of fire aims at stimulating regrowth of grasses for cattle, fire and cattle grazing are a common combination, and together they have catastrophic effects on the population structure of *Polylepis* woodlands (Fjeldså & Kessler 1996). In areas with high stocking rates, a reduction in livestock density reduced browsing on *P. australis* individuals (Teich *et al.* 2005). *P. australis s*eed tree cover was higher in areas with low stocking rate compared to areas with high stocking rate (Torres *et al.* 2008). Seedling survival, growth per year, and density in *P. australis* was higher in the less grazed areas or in places with low stocking rate (Teich *et al.* 2005; Torres *et al.* 2008). Moreover, the size structure changed in the heavy grazed areas, which had fewer individuals between 60 and 200cm in height (Teich *et al.* 2005). Thus, when comparing extreme conditions, cattle had clear negative effects on *P. australis* regeneration in Argentina. However, when intermediate animal loads were present, a different pattern was observed. *P. australis* seedling occurrence was highest under moderate grazing intensities, compared to the places with grazing exclusion or high stocking rate (Zimmermann *et al.* 2009). In Ecuador, there was no evidence of negative effects of moderate cattle grazing on *P. incana* and *P. pauta* (Cierjacks *et al.* 2008a); in this case, trampling had a positive impact on seedling abundance, likely due to the removal of the litter layer and thus the creation of safe-sites for the establishment of the species.

Fire and cattle effects on *Polylepis* woodlands threaten these ecosystems mainly in the long term. In the short term, many fragments are threatened by overcutting, as the expansion of roads and industrial development projects brings new people to areas previously less accessible and less populated (Purcell & Brelsford 2004). In fact, these authors have found a loss of 87% of the 235ha previously reported for three sectors in the eastern Bolivian Andes, likely caused by clearcut in a 12yr (1991-2003) period. In Peru, distance to the nearest village, road and market centre, explained the regeneration of five *Polylepis* species, after statistically removing the effect of elevation in humid and dry places (Toivonen *et al.* 2011).

Despite the impact of the introduction of exotic species on native vegetation (Stohlgren *et al.* 1999; Ni *et al.* 2010), habitat fragmentation (Fischer & Stöcklin 1997; Barbeta *et al.* 2011) and global warming effects (Pounds *et al.* 1999; Parmesan 2006) on mountain woodlands, only few studies addressing these issues were found for the high Andean *Polylepis* woodlands. The effects of fragmentation on the genetic variability of *Polylepis* species from Ecuador and Argentina have only recently been studied (Julio *et al.* 2008; Aragundi *et al.* 2011; Julio *et al.* 2011; Hensen *et al.* 2011; Hensen *et al.* 2012) and are discussed in detail in Chapter 7. *P. australis* woodland fragmentation did not affect pollen loads on the stigmas of flowers nor reduced its seed viability (Renison *et al.* 2004; Seltmann *et al.* 2007b), suggesting that pollen limitation is not a main factor acting on *Polylepis* reproduction. Although the species has the potential for inbreeding depression, pollen flow between *P. australis* woodlands seems still effective enough to counteract negative effects on their seed quality in Argentina (Seltmann *et al.* 2009). Increasing fragment size increased reproductive success measured as leaf area and biomass of *P. australis* seedlings (Seltmann *et al.* 2007b). Finally, only one study analyzed the performance of *P. australis* along its altitudinal range, and found that any rise in temperature will further restrict *P. australis* to its uppermost distributional altitudes (Marcora *et al.* 2008).

Polylepis woodlands in Bolivia

Bolivia is one of the core areas for the genus' diversity, it contains 13 out of the *c.* 26 species of *Polylepis* (Kessler & Schmidt-Lebuhn 2006) and nine are considered vulnerable (Chapter 2). In the nineties, the potential cover of *Polylepis* has been estimated to be 51,000km^2 (20%) of the Bolivian highlands (Kessler 1995) and their cover was estimated as about 11% of that area: 100% of the potential area on the arid western Andean range, 0.02% on the central high plateau and Titicaca basin, and 1.44% on the eastern highlands (Kessler 1995). An assessment of 17 woodlands in the latter region revealed a loss of 87% of the original described area (235ha) and a loss of 41% of all woodland remnants in only 12 years (Purcell & Brelsford 2004). The main causes for the severely reduction of *Polylepis* area in Bolivia were identified as the extensive grazing and fire (Kessler 1995; Kessler & Herzog 1998), as well as overcutting because of increased accessibility due to road building and mining (Purcell & Brelsford 2004). No information was found on the effects of fragmentation and climate change on Bolivian *Polylepis* species, and only one study addressed the effects of exotic tree species on *Polylepis* (Larrazabal 2004).

Polylepis besseri Hieron. and *P. subtusalbida* (Bitter) M. Kessler & Schmidt-Leb are two of the threatened species inhabiting the high Andes in the departments of Cochabamba and Chuquisaca. Formerly, *P. subtusalbida* was considered a subspecies of *P. besseri*, but it became a ‘full’ species because of a reassessment of species limits in *Polylepis*, which considered morphologically, biogeographically and ecologically distinct populations at species rank, even if there is evidence for hybridization (Kessler & Schmidt-Lebuhn 2006). *P. subtusalbida* presents more langish leaflets, a grayish tone to the upperside of the leaflets, and less white woolly hairs on the flowering stands compared to *P. besseri* (Fjeldså & Kessler 1996). Both species occupy the Tunari zonal punean woodlands in the Peruvian Puna biogeographic province (Navarro & Maldonado 2002), thus share the pluviseasonal subhumid bioclimate. However, the *P. besseri* woodlands have a rather more southerly distribution and is more closely linked to the Bolivian-Tucuman biogeographic province than the *P. subtusalbida* woodlands (Navarro *et al.* 2005). *P. besseri* woodlands in Sacha Loma and Cuturi (17°44’S, 65°34’W, Cochabamba) were among the largest, well delimited, and most dense *Polylepis* woodlands left within a 60km range from the city of Cochabamba (Cahill & Matthysen 2007), and thus scientific knowledge regarding its conservation is crucial. *P. subtusalbida* woodlands located in the Parque Nacional Tunari (17°20’S, 66°10’W, Cochabamba) - created in 1962 to protect native vegetation, prevent soil erosion, provide protection against the development of gullies around the city of Cochabamba, and as recreational space (Rico 1996; Anon. 2001) - are one of the most representative native woody vegetation in the highlands above the city of Cochabamba.

Given the economic and ecological importance of these high Andean ecosystems, their potential much more extended distributional range in Bolivia, their threatened status, and their uncertain fate under climate change, scientific knowledge about the response to its threats is extremely important for developing a sound conservation and restoration strategy. This PhD work aims at 1) describing the species diversity inhabiting *Polylepis* woodlands in Bolivia; and 2) studying how *Polylepis* trees and populations may respond to three major threats: global warming, introduction of exotic tree species, and habitat fragmentation (Fig. 1.2). In the last chapter (Chapter 8) we discuss practical guidelines for the conservation of *Polylepis* species in Bolivia.

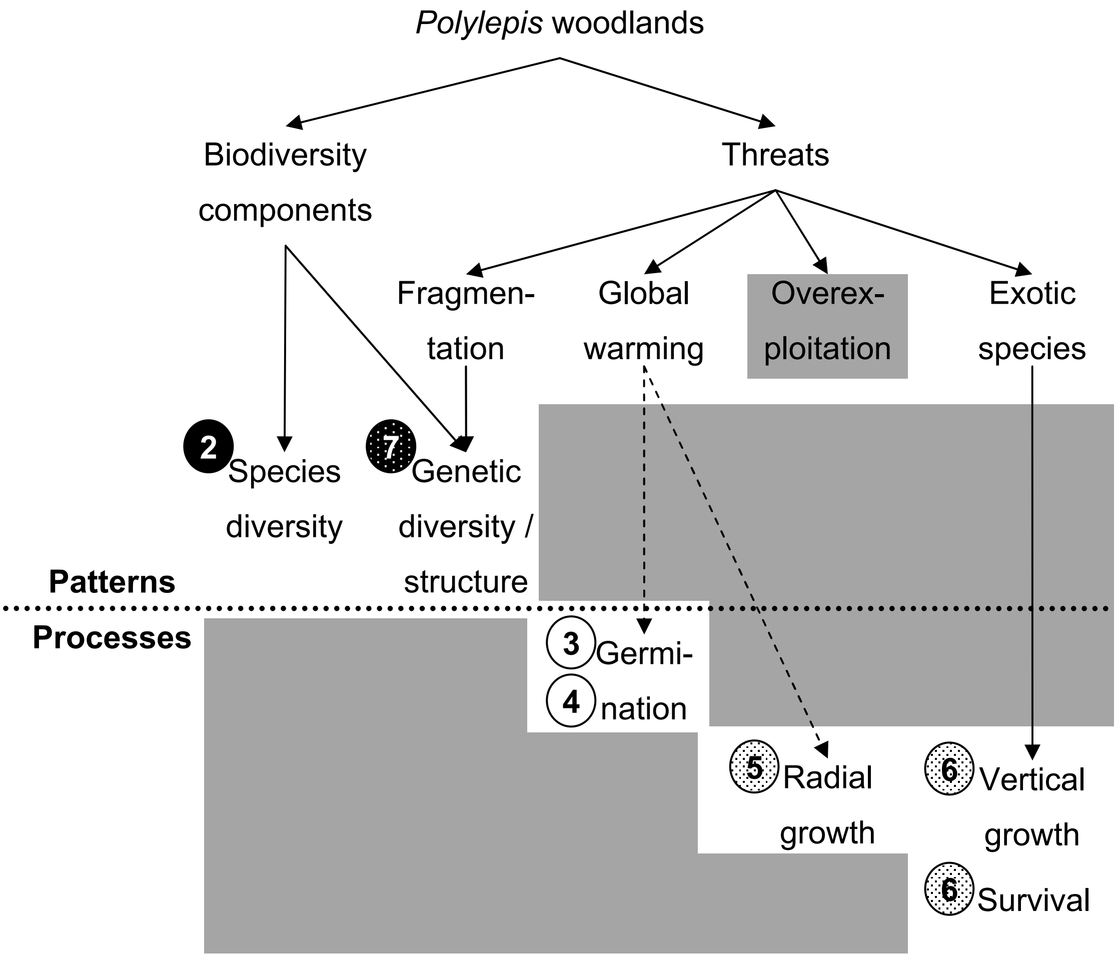

Fig. 1.2 Schematic representation of the *Polylepis* woodlands conservation problem addressed in this study. *Numbers in circles* represent chapter numbers. *Black circles* represent literature reviews, *white circles* represent lab and field experiments, and *dotted circles* are observational studies. *Segmented lines* indicate indirect evidence. *Gray areas* represent potential research not studied in this work (see Fig. 8.1).

This study is limited to monospecific *Polylepis* woodlands of *P. besseri* and *P. subtusalbida* in the dry eastern Bolivian Andes in Cochabamba – Bolivia, one of the priority areas for *Polylepis* conservation because of a high concentration of endemic species, and because soil and water conservation are becoming critical factors in many places (Fjeldså & Kessler 1996), including the city of Cochabamba. Typical monospecific woodlands of *P. besseri* and *P. subtusalbida* were selected as study subjects. Our approaches include literature reviews (Chapters 2 and 7), observational studies (Chapters 5, 6 and 7) and field and laboratory experiments (Chapters 3 and 4) (Fig. 1.2).

Polylepis woodland biodiversity in Bolivia

Given the lack of a review on *Polylepis* woodlands diversity, the availability of a large amount of literature on this topic, and their importance for biodiversity conservation, we reviewed the knowledge on the species diversity for the Bolivian *Polylepis* woodland remnants, assessed the conservation status of the occurring species, determined their trophic niche, and related species richness with climatic variables and elevation (Chapter 2). This chapter serves as background chapter and an umbrella for other more detailed studies as it enables to find the most important knowledge gaps regarding biodiversity in Bolivian *Polylepis* woodland remnants.

Regeneration ecology of *P. besseri* under both lab and field conditions and inferences regarding climate warming and exotic species effects

Forest regeneration starts with the production of seeds and its germination, and includes seed and sapling growth and survival. Seed germination is a crucial event in a plant's life cycle because it determines the future conditions that plants will experience. We studied both lab (Chapter 3 & 4) and field germination (Chapter 4). Because temperature and water availability are important regulators of seed germination, this process will likely be influenced by global warming. As hydrothermal time (HTT) models indicate that the timing of germination is closely related to temperature and water potential thresholds for radicle emergence which vary among individual seeds in a population (Welbaum *et al*. 1998), they can be used to evaluate seed germination for any combination of water potential and temperature, and therefore be suitable to predict global warming effects on seed germination. We studied the germination characteristics of the high Andean endemic tree species *Polylepis besseri*, using HTT models (Chapter 3). We were especially interested in the potential effects of global warming on seed germination.

Species from mountain ecosystems are expected to be very susceptible to global warming, and the options they have to survive under a warmer climate include to be pre-adapted, to move or to adapt. In this context, reciprocal transplant experiments across an altitudinal gradient, allow the estimation of local adaptation and of effects of global warming on plant populations. Moreover, under the extreme abiotic conditions in the high Andes plant facilitation may play an important role in the germination. We combined reciprocal transplant experiments and HTT germination models to detect local adaptation of *P. besseri* seed characteristics to altitude (Chapter 4). We asked the following questions: 1) how is seed germination success changing when seeds are introduced at different altitudes? 2) Is facilitation the dominant plant-plant interaction at different altitudes? 3) Do seeds from different altitudes have different germination requirements as quantified by their HTT model parameters?

Growth and survival of *Polylepis* seedlings are another aspect of *Polylepis* regeneration. The establishment of exotic trees such as *Pinus radiata* (radiata pine, Pinaceae) and *Eucalyptus globulus* (eucalyptus, Myrtaceae) in fragments of native vegetation at the Parque Nacional Tunari (PNT, Cochabamba-Bolivia), can affect the regeneration of the native species that live there. *P. subtusalbida*'s regeneration was surveyed, evaluating the density of seedlings, their size structure, and growth forms, as well as variables of growth and survival in fragments of pure forests (*P. subtusalbida* fragments without exotic trees) and mixed fragments (*P. subtusalbida* fragments with exotic trees). Because of the ecological and morphological similarities between *P. subtusalbida* and *P. besseri*, we think the results we obtained are relevant for both species (Chapter 5).

Regional climate *vs*. radial growth relationships for *P. besseri*

High-altitude *Polylepis besseri* woodlands in Bolivia are under increasing threat from human use and disturbance. Currently, there is no information regarding *P. besseri* growth rates, age structures or the relationship between environmental variables and growth. Such information would be useful for effective management and conservation of the remaining woodlands, including the potential effects of climate change. We used standard dendrochronological techniques to determine the age and radial growth rates for 23 trees from two *Polylepis besseri* populations in Sacha Loma (mountains Uypa and Chutu Senega), and investigated the relationship between climate and radial growth (Chapter 6).

Population genetics of fragmented *Polylepis* woodlands

There is a long-standing debate on whether the occurrence of the high Andes *Polylepis* woodlands as small and isolated fragments in a grassland matrix is of natural or of anthropogenic origin. We make inferences regarding their fragmentation history, based on an additional population genetic study on *P. besseri* in Bolivia, and a synthesis of available studies investigating the population genetic variation of *Polylepis* woodlands (Chapter 7). We infer the timing of the main woodland fragmentation event through the interpretation of 1) levels of population genetic diversity, and their relation with population size; 2) population genetic differentiation and 3) comparison of the population genetic diversity between the offspring and the adult generation.

General discussion & conclusions

Finally, we discuss conservation and restoration consequences of our findings for this important high Andean genus (Chapter 8).

CHAPTER 2

Polylepis woodland biodiversity in Bolivia

Adapted from Gareca, E.E., M. Hermy, J. Fjeldså & O. Honnay. 2010. *Polylepis* woodland remnants as biodiversity islands in the Bolivian high Andes. *Biodiversity and Conservation* 19: 3327-3346.
The original publication is available at www.springerlink.com

Introduction

Mountain forests deserve special attention from ecologists and conservation biologists because they offer a variety of ecological services to humans (Douglas 1999; Sidle *et al.* 2006) and they are important biodiversity hotspots (Burgess *et al.* 2007; Menegon *et al.* 2008). It has been suggested that mountain ecosystems could be particularly prone to changes in species composition through global change, with consequences for the ecosystem services (Kräuchi *et al.* 2000; Schröter *et al.* 2005; Williams *et al.* 2007), although it has also been suggested that "hotspots of endemism" in tropical mountains may include exceptionally stable local environments (*e.g.*, Fjeldså & Irestedt 2009).

The Andes in South America forms a nearly continuous high-altitude mountain chain in the western part of the continent and contains the largest extension of low-temperature areas within the tropical belt of the world (Sarmiento 1986). The ecosystems above the forest limit in the tropical Andes in South America consist of a mosaic of low grass and scrub with occasional oases of more dense vegetation mainly dominated by monospecific stands of one arborescent genus, *Polylepis*. However, these mosaic landscapes are present in a handful of extensive pristine timberline areas where grazing or burning are absent, while in most of the highland these practices have now lead to a monotonous bunchgrass terrain with an abrupt ecotone from closed forest to scrub or grassland (Kessler & Herzog 1998; Jácome *et al.* 2007). As other mountain forests, these tropical subalpine *Polylepis* woodlands provide valuable ecosystem services (Fjeldså & Kessler 1996; Fernández *et al.* 2001). Because *Polylepis* woodlands are such an important supply of resources (wood for fuel, tools and house construction, medicinal and nourishing plants) for local people (Fjeldså & Kessler 1996; Kessler 2006), as well as because of the extensive grazing and burning practices, *Polylepis* woodlands have been severely diminished and they are considered as one of the most threatened ecosystems in South America (Kessler 1995; Herzog *et al.* 2002; Navarro *et al.* 2005).

Most of *Polylepis* woodlands are isolated patches in landscapes dominated by grasslands, and they are often found on rocky slopes or along streams (Simpson 1979; Fjeldså & Kessler 1996). Early explorers considered these marginal sites as their natural distribution area (*e.g.* Herzog 1923, Troll 1929 in Kessler 2002). More recent evidence, however, supports Ellenberg's (1958) hypothesis that the patchy distribution of *Polylepis* woodlands is mainly due to thousands of years of human influences (see also Kessler 2002), and that the climax vegetation of parts of the Andean region actually is *Polylepis* woodland (Fernández-Terrazas 1997).

Bolivia is a core area for the genus' diversity, containing 13 out of the *c.* 26 species of *Polylepis* (Kessler & Schmidt-Lebuhn 2006). In total, *Polylepis* could cover about 51,000km^2 (20%) of the Bolivian highlands (Kessler 1995); however, in the nineties it only covered about 11% of this area: 100% of their potential area in the arid western Andean range, 0.02% in the central high plateau and Titicaca basin, and 1.44% in the eastern highland (Kessler 1995). Moreover, a recent assessment of the latter region after 12yr revealed that 10 out of 17 sites re-evaluated remained, this represents a loss of 41% of woodlands and 87% of the 235ha reported for the period 1991-1993 (Purcell & Brelsford 2004). Twenty out of the *c.* 26 described *Polylepis* species have been categorized as threatened (IUCN 2008). Given the economical and ecological importance of these high Andean ecosystems, their potential extended distribution, their threatened status, and their uncertain fate under climate change, conservation, monitoring and restoration of *Polylepis* woodland remnants are extremely important.

The primary aim of this study was to present a review of the knowledge regarding the species diversity of the Bolivian *Polylepis* woodland remnants, and to investigate their conservation status. We also aimed at relating species richness with climatic variables and elevation, and at identifying the most important knowledge gaps regarding the biodiversity of *Polylepis* remnants in Bolivia.

***Polylepis* woodlands**

The genus *Polylepis*

The description of the life form, flowers, and fruits of the genus *Polylepis*, as well as the description of the physiognomy of their woodlands, can be found in Chapter 1.

Phylogenetic analyses suggest that *Polylepis* evolved via polyploidy from *Acaena*, a genus of herbs and subshrubs (Fjeldså & Kessler 1996; Kessler 2006). Radiation and specialization appears to have occurred northward and southward from the vicinity of northern Peru and to higher elevations (Simpson 1979). The tree form might have evolved since the middle of the Miocene (*c.* 15mya) when the Andes first reached 1500m (Fjeldså & Kessler 1996). As the Andes rose, species of *Polylepis* apparently spread along the eastern slopes of the Andes and across the mountain ranges where there was enough moisture for woody vegetation (Simpson 1986). The most primitive *Polylepis* species occur in the humid forest in the Yungas region (Fig. 2.1a). The subsequent evolution reduced the number of leaflets and flowers, increased the thickness of leaves and the presence of resins or hairs on leaves, and reduced the tree size; all of these helped surviving the dry and cold conditions of the Pleistocene (Fjeldså & Kessler 1996; Schmidt-Lebuhn *et al.* 2006). It is very likely that the evolution of the genus was mainly governed by climate fluctuations during this epoch, forcing the migration of the species to places with favourable ecological conditions, and therefore fragmenting the species distribution (Fjeldså & Kessler 1996; Kessler 2006).

Given the potential distribution proposed for *Polylepis* in Bolivia (Kessler 1995), the fact that trees are functional throughout the year, and that most *Polylepis* species are exposed to night frost (frequent in the Andes) and diurnal temperature variations that by far exceed seasonal ones (Goldstein *et al.* 1994), one of the most important attributes for the survival of most species of the genus is frost tolerance. There might be considerable variation in this trait, since species which are basal in the phylogeny generally grow in frost-free cloud-forest habitats. Additionally, even on high elevations, the interior of dense *Polylepis* forests are generally frost-free (even if areas right outside the forest are covered by snow). Although the species need to cope with frost at night in order to colonize new environments, likely it concerns only few species representing terminal branches in the phylogeny that are adapted to endure strong frost. Comparing this trait between a primitive species (*P. sericea*) and more recent ones (*P. australis* and *P. tarapacana*), Azócar *et al.* (2007) showed that *P. sericea* presented frost avoidance through supercooling capacity (*i.e.* injury and freezing temperatures between -8 to -9°C). Whereas *P. australis* and *P. tarapacana* presented frost tolerance (*i.e.* injury temperatures, -18 to -24°C, and freezing temperature, -3.5 to -9.2°C). Thus, this trait may have evolved from frost avoidance to frost resistance. These results coincide with the proposed evolutionary history of the genus indicating an adaptation to cold conditions (Simpson 1986; Fjeldså & Kessler 1996).

Polylepis species in Bolivia

There are 13 *Polylepis* species and three subspecies in Bolivia (Table 2.1), all of them in the Andean region (Kessler & Schmidt-Lebuhn 2006). This region can be subdivided into four biogeographic provinces (Navarro & Maldonado 2002): the Peruvian Puna province and the Andean Altiplanic province in the inner parts of the highlands, and the Peru-Bolivian Yungas

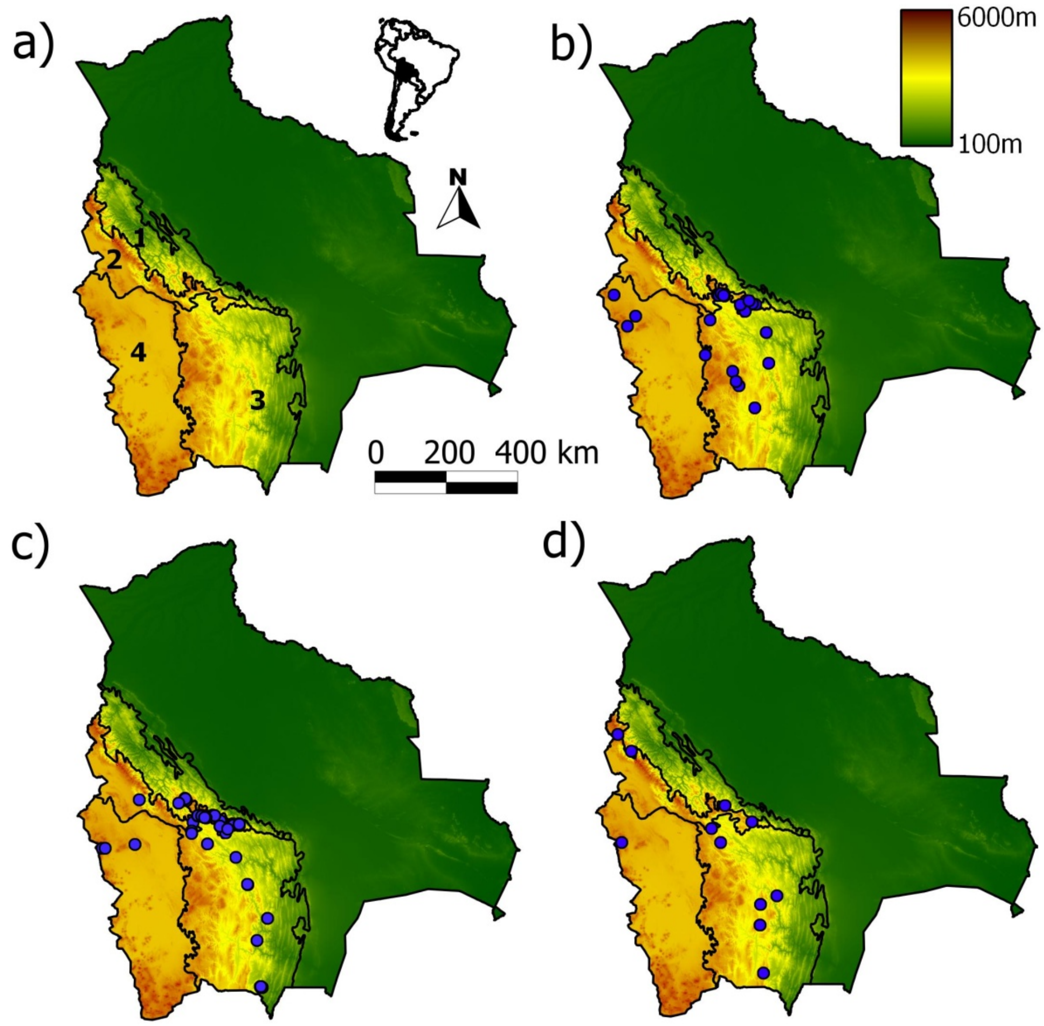

Fig. 2.1 a) Location of the four Andean Biogeographic Provinces of Bolivia. *1*: Peruvian-Bolivian Yungas, *2*: Peruvian Puna, *3*: Tucuman-Bolivian and *4*: Altiplanic Biogeographic Province (after Navarro and Maldonado 2002). *Blue dots* are locations with adequate data for the comparison with climatic variables concerning plant biodiversity (b), bird biodiversity (c) and mammal biodiversity (d). Gray intensities represent topography: *Green* are elevations close to 100m, *yellow* represents 3000m, and *brown* 6000m.

and the Bolivian-Tucuman provinces in the transition to the Bolivian lowlands (Fig. 2.1a).

The Andean Altiplanic province has a xeric and pluviseasonal bioclimate and an altitude ranging between 3100m and more than 5200m. *Polylepis tarapacana* forms dispersed bushy woodlands with associated vegetation of *Mutisia lanigera* at altitudes between 3900m and 4800m, and some *P. tarapacana* individuals occur above 5000m (Navarro *et al.* 2005).

The Peruvian Puna has mainly a pluviseasonal climate, it includes altitudes between 2000m and above 5200m (Navarro & Maldonado 2002), and seven species of *Polylepis* are found in this province (Table 2.1). The characteristic communities and distribution of *Polylepis subtusalbida* (with *Berberis commutata*), *P. lanata* (with *B. paucidentata*), *P. tomentella* subsp. *nana* and *P. tomentella* subsp. *incanoides* (both with *B. rariflora*), and *P. besseri* are described in Navarro *et al*. (2005). *Polylepis incarum* is potentially distributed between 3400-4000m in the Cordillera Real and Titicaca zone, although it is now represented by very dispersed and small groups of trees (Navarro & Maldonado 2002). *P. pacensis* (3250-4130m) and *P. pauta* (3500-3550m) are found on the most humid grassland on the transition between the Peruvian-Bolivian Puna and the Yungas slopes (Kessler & Schmidt-Lebuhn 2006).

The Peru-Bolivian Yungas province (500-4200m) presents xeric to pluvial bioclimates (Navarro & Maldonado 2002). Four *Polylepis* species occur (Table 2.1), and the characteristic communities of *Polylepis pepei* (with *Gynoxis asteroricha*), *P. lanata* (with *Ilex mandonii*, *Styloceras columnare* and *Berberis edentata*), and *P. triacontandra* (with *Styloceras columnare*) are described in Navarro and Maldonado (2002). *P. sericea* is found sometimes as tall forests in the Yungas at 2950-3700m. Finally, the characteristic communities of the *Polylepis* species found in the Bolivian-Tucuman region, mainly with pluviseasonal and xeric bioclimates (500-above 5200m), are described for *P. tomentella* subsp. *tomentella* (with *Azorella compacta* and *Dasyphyllum hystrix*) and *P. neglecta* (with *Schinus microphyllus*) in Navarro *et al*. (2005), and for *P. hieronymi* (with *Alnus acuminata*) and *P. crista-galli* (with *Escallonia hypoglauca*) in Navarro and Maldonado (2002).

In summary, the high Andes of Bolivia holds species belonging to the most primitive, as well as the most evolved groups of *Polylepis*. The basal group is represented by *P. sericea* and *P. pepei*, which occur in the pluvial bioclimate (Peru-Bolivian Yungas). The phylogenetically terminal species occur in the Peruvian Puna (pluvi-seasonal bioclimate), Bolivian-Tucuman (pluvi-seasonal and xeric bioclimates), and Altiplanic provinces (xeric bioclimate). Thus, Bolivia is a key area to the genus and offers the different adaptations that occurred during the evolution of these high mountain trees.

Table 2.1 *Polylepis* species found in Bolivia, endemism, and IUCN category.

Polylepis species	Biogeographic Province[a]	Endemism[b]	Conservation status[c]
P. tarapacana	Altiplanic	B, P,Ch	NT
P. besseri	Puna	B,P[d]	V
P. incarum	Puna	B, P	V
P. pacensis	Puna	B	
P. pauta	Puna	B, P, E	
P. subtusalbida	Puna	B	V
P. lanata	Puna, Yungas	B	V
P. pepei	Yungas	B, P	V
P. sericea	Yungas	B, P, V, E, C	
P. triacontandra	Yungas	B, P	V
P. crista-galli	Tucuman	B, A	V
P. hieronymi	Tucuman	B, A	V
P. neglecta	Tucuman	B	V
P. tomentella ssp. *incanoides*	Tucuman, Puna	B	V
P. tomentella ssp. *nana*	Tucuman, Puna	B	CE
P. tomentella ssp. *tomentella*	Tucuman, Puna	B, A	NT

[a] *Puna*: Peruvian Puna, *Yungas*: Peru-Bolivian Yungas, *Altiplanic*: Andean Altiplanic, *Tucuman*: Bolivian-Tucuman. Following Navarro and Maldonado (2002) and Navarro *et al*. (2005) and estimated based on the coordinates given by Fjeldså and Kessler (1996) and Kessler and Schmidt-Lebuhn (2006).
[b] *B*: Bolivia, *P*: Peru, *Ch*: Chile, *E*: Ecuador, *V*: Venezuela, *C*: Colombia, *A*: Argentina
[c] *NT*: Near threatened, *V*: Vulnerable, *CE*: Critically endangered, based on the IUCN Red List (www.iucnredlist.org, March 2009)
[d] A population in the Urubamba Valley of Cuzco, Peru was placed in *P. besseri* by Simpson (1979, cited in Kessler & Schmidt-Lebuhn, 2006)

Endemism and conservation status of *Polylepis* species in Bolivia

Four species and two subspecies of the genus *Polylepis* are endemic to Bolivia (Table 2.1); they are located in all the Andean biogeographic provinces, suggesting that new adaptations occurred for all these environments. Four of these endemic species are considered vulnerable (Table 2.1), denoting the need for research, conservation and restoration to be taken in Bolivia. The other nine non-endemic species of the genus in Bolivia are shared mainly with Peru or Argentina (Table 2.1). Concerning the conservation status of the Bolivian *Polylepis* species, nine of the 13 species are considered vulnerable, and one is near threatened (Table 2.1). Bolivia has one *Polylepis* subspecies near threatened, another vulnerable and one is considered critically endangered by the IUCN red list of threatened species (Table 2.1).

Methods

In January 2009 we first performed a search in the ISI web of Knowledge with the search terms: "*Polylepis*", "*Polylepis*" AND "Andes", and "*Polylepis*" AND "tree". A second search was made in the Net of Latin-American, Caribbean, Spanish and Portuguese journals, using only the search term "*Polylepis*". Finally, specific searches were made in the journal Ecotropica (holder of the papers of the 1st International Congress on *Polylepis,* 1995-2008), Ecología en Bolivia (August 2001-2008) and the Revista Boliviana de Ecología y Conservación Ambiental (1997-2008) using "*Polylepis*" as the only search term. Admittedly, this kind of search is far from complete, as it overlooks the wealth of information that is scattered in older literature describing Andean habitats (*e.g.*, Troll 1929 and his many other papers on timberline habitats), or which is "buried" in numerous survey reports ("grey literature"), faunistic papers and taxonomic revisions, where habitats were not specified in the list of keywords.

Because of the very large data set we collected from literature, systematic ground truthing was not feasible. We looked for publications with species lists of *Polylepis* woodland remnants in Bolivia or with *Polylepis* species in their list. Then, we compiled species lists of bryophytes and vascular plants that occur in woodland remnants where *Polylepis* is the dominant tree species. Plants were classified by growth form. Birds, mammals, reptiles and Lepidoptera were classified by their trophic niche. Amphibians were not included in this review because they are mainly linked to water sources and are not as dependent on *Polylepis* woodlands as the other organisms. The species lists with the names presented by the authors, their guild or growth form and their conservation status (see below) are in

Appendices 1 and 2. Next, in order to explore the development in knowledge about *Polylepis* woodland remnants in Bolivia, we created species accumulation curves through time using the time of the survey in the *x* axis, starting with the oldest published field work, and ending with the most recent.

Based on the published or derived coordinates of each study location (Appendix 3), local climate variables were retrieved from the Local Climate Estimator New Loc Clim v. 1.10 (Grieser *et al.* 2006). These included maximum, mean and minimum temperature (°C), annual precipitation (mm), potential evapotranspiration (mm) and length of the growth season (days). These variables were estimated using Sheppards method and gradient correction (vertical and horizontal, Grieser *et al.* 2006). Elevation of the study sites was added to the dataset if reported; otherwise it was obtained from Google Earth. For the birds, new survey data were added based on data gathered during earlier expeditions by Jon Fjeldså and his field teams. This new data and nine other studies from which it was possible to retrieve the coordinates and the elevation, and that assigned species to a specific locality, were used for studying the relationship between climate and species richness (Appendix 3). Additionally and only for the birds, the habitat quality, defined as good or poor, was added based on the descriptions made by the authors and Jon Fjeldså's personal observations (descriptions for poor quality sites: degraded woodland, relict, scrub, mixed woodland, exotic tree plantations, high human impact; descriptions for good quality sites: substantial woodlands, distinguishable patches, dense vegetation, large areas, low or moderate human impact), and endemism, measured as the inverse of the range-size for each species, was averaged per location for all the species and for the 25% of the species with smallest ranges. The database of bird range-size was developed mainly by J. Fjeldså, this included first of all data from Birds of the High Andes (Fjeldså & Krabbe, 1990) and all literature sources therein, but has been currendly updated by later publications and even trip records (scrutinized through communications with data providers if they contained suspect records) and J. Fjeldså's field observations. As a basis of interpolation for grid cells between those with records, inspection of vegetation and landscape structure from Google earth (and based on J.

Fjeldså's travels through many areas) was used (see Fjeldså & Irestedt, 2009). Both, mean endemism and species richness per location were related to the local climate variables using Spearman rank correlations in STATISTICA 6.0 (StatSoft Inc. 2001). To determine the effect of habitat quality on endemism, a covariance analysis was performed using potential evapotranspiration as a covariate. Finally, for the animals, the conservation status of the occurring species in the woodlands was obtained from the IUCN Red List (IUCN 2008), and for the plants, from the latter as well as from Meneses and Beck (2005).

Results

Our search yielded 140 papers mentioning the Andean tree *Polylepis*. The first paper found in the ISI web of knowledge was a review about the pollen morphology of *Polylepis* (Smit 1978). Since then, the number of publications increased with time, and 52 papers were found in our search in the period 2006-2008. During the whole period (Fig. 2.2a), most of the publications were in the ISI web of Knowledge database (63%), followed by publications in Bolivian journals (20%) and Latin American journals plus Ecotropica (17%). The most important input in the latter category took place the year 2002 with the papers of the 1st International Congress on *Polylepis* held in Bolivia in 2000 (Fig. 2.2a; Herzog *et al.* 2002). Twenty four of the retrieved studies dealt with plant and animal species diversity of Bolivian *Polylepis* woodland remnants (Fig. 2.2b) and next to seven additional documents (2 guides, 1 book, 3 reports and 1 thesis), were used for subsequent analyses (31 publications).

Cumulative species richness

Data on associated plant species richness were found for seven out of the 13 species and three subspecies of *Polylepis* occurring in Bolivia (*P. lanata*, *P. subtusalbida*, *P. tomentella* subsp. *incanoides*, *P. tomentella* subsp. *nana*, *P. besseri*, *P. tarapacana* and *P. neglecta*). Three of these species include data for the bryophyte species richness (*P. lanata*, *P. subtusalbida* and *P. tomentella* subsp. *nana*).

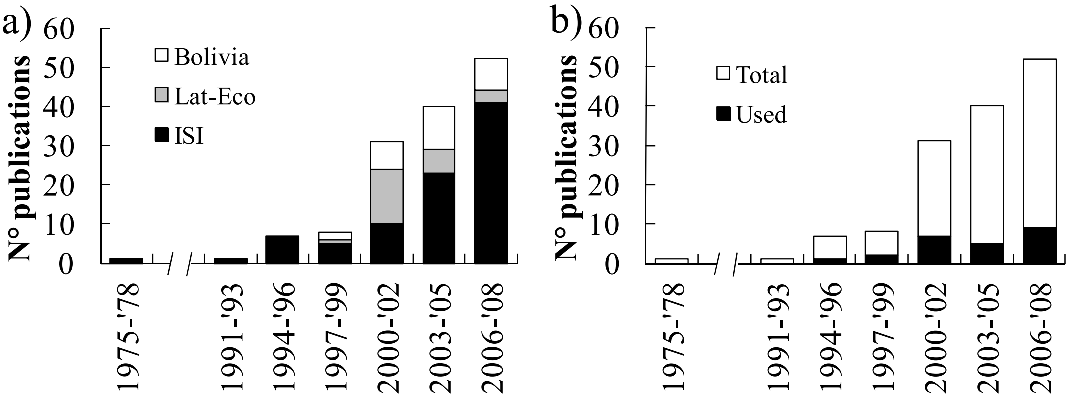

Fig. 2.2 a) Number of publications found in a literature survey in January 2009 with the search terms "*Polylepis*" in the ISI web of knowledge (*black area*), in Latin American journals and Ecotropica (*gray area*) and in Bolivian journals (*white area*). b) Number of publications used for the analyses presented in this review. For details see text.

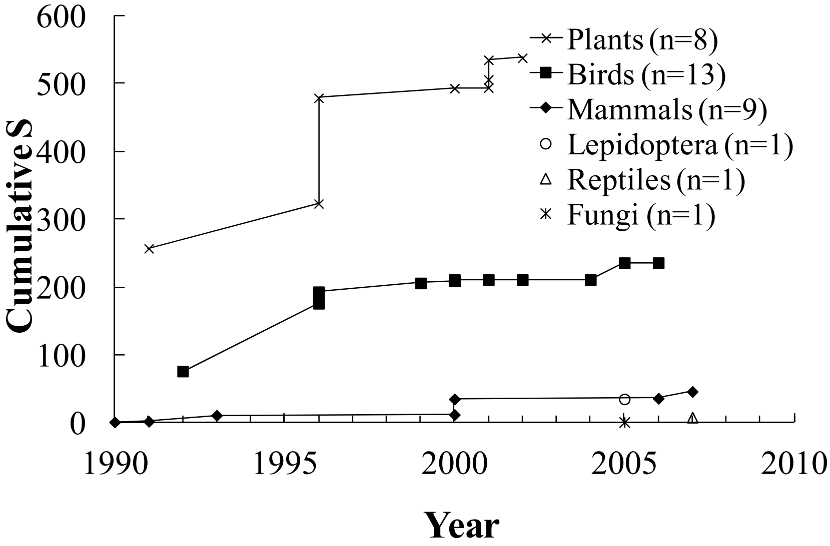

Fig. 2.3 Cumulative species richness of the organisms reported for the *Polylepis* woodland remnants. The numbers of publications with suitable data are in parenthesis.

The six *Polylepis* species with data of associated plant species richness occur in the four biogeographical provinces of the Bolivian Andes (Fig. 2.1b). Most of the lists were from the Cochabamba region (Fernández-Terrazas 1997; Mercado-Ustariz 1998; Fernández *et al.* 2001; Balderrama & Ramirez 2001; Navarro 2001; Hensen 2002). Two studies list plants from other departments (Mueller *et al.* 2002; Navarro *et al.* 2005), increasing the cumulative species richness found in the *Polylepis* woodland remnants with 6%. The species accumulation curve for plants (Fig. 2.3) did not level off. The first study (1990-1991) reports 257 species of plants from *Polylepis* woodland remnants in the Cochabamba region (Hensen 2002). The second major input to the cumulative plant species richness, also performed in the Cochabamba region, described 254 species (Fernández-Terrazas 1997; Fernández-Terrazas & Ståhl 2002), of which 155 were not described in previous work. The most species rich families were the Asteraceae (101 spp.), followed by the Poaceae (50 spp.) and Scrophulariaceae (17 spp.); ferns were also abundant (57 spp.).

The cumulative bird species richness was almost half of that of the plants (266 species, Fig. 2.3). The shape of the species accumulation curve is more flattened than for the plants (Fig. 2.3) and Fjeldså and Kessler (1996) include most of the birds now known for this habitat (169 species). Another major input comes from the Parque Nacional Tunari in Cochabamba (Balderrama 2006) containing 150 species and adding 11% to the cumulative species richness for the *Polylepis* woodland remnants. Most of the additional species are best regarded as rare visitors from forests at lower elevations.

Tarifa and Yensen (2001) performed the major contribution to the mammal species list (Fig. 2.3); they reported 30 species of which 23 were new for the *Polylepis* woodland remnants. Their collection was made all over the country (Fig. 2.1d). The second major input was collected for Parque Nacional Tunari in Cochabamba (Aguirre *et al.* 2007), containing 17 species, and adding 22% to the cumulated mammal species richness for the *Polylepis* woodland remnants.

Finally, there is only one study on Fungi (Macía *et al.* 2005), one on butterflies (Lepidoptera, section Rhopalocera) (Quinteros *et al.* 2006) and one on Reptiles (Aguayo *et al.* 2007). These three studies are quite recent and less than 40 species were recorded per group (Fig. 2.3). However, several recent taxonomic revisions of other Lepidoptera groups ("microlepidoptera" and moths, see Fjeldså and Kessler 1996) described numerous species which are narrowly associated with *Polylepis* habitats, as indicated by their striking adaptations for crypsis when they rest on the reddish *Polylepis* bark. In total, we built a list of 537 plants (425 identified to the species level), 266 birds, 46 mammals, 35 butterflies and 8 reptiles (Appendix 1 and 2).

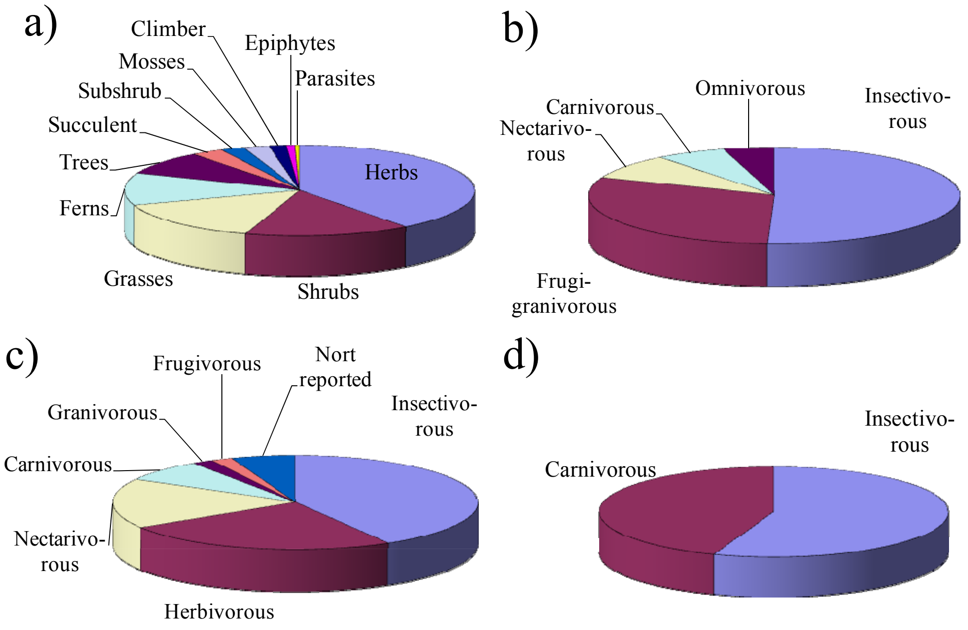

Fig. 2.4 Percentages of guilds for the a) plants (n=537, growth form), b) birds (n=266), c) mammals (n=46), and d) reptiles (n=8).

Growth form and trophic niche

The growth form was assigned to 502 plants (93%). Most of the plants found in the *Polylepis* woodland remnants were herbs (40%), followed by shrubs, grasses, ferns, trees, succulents, and others (Fig. 2.4a, Appendix 1). Fifty one percent of the birds were insectivores, followed by the frugi-granivorous, nectarivorous, carnivorous and omnivorous species (Fig. 2.4b, Appendix 2). Also insectivorous mammals were the most abundant (47%); followed by herbivorous, nectarivorous, carnivorous and frugi-granivores (Fig. 2.4c, Appendix 2). The reptiles were insectivorous and carnivorous (Fig. 2.4d, Appendix 2). The adult butterflies are nectarivorous (18 out of 35 species reported for the *Polylepis* woodland remnants, Appendix 2), although their larvae are herbivorous and generally associated with the rich herbaceous vegetation of these forests rather than with the *Polylepis* trees.

Conservation status of the species

Most of the birds (97%) and mammals (85%) recorded for *Polylepis* woodland remnants were considered in the IUCN red list of threatened species, whereas no reptile or butterfly species were evaluated by the IUCN (IUCN 2008). In total, 14 species have been categorized as threatened (vulnerable or endangered, Table 2.2): 7 plant species, 4 bird species and 3 mammal species; whereas 11 species were categorized as near threatened: 10 birds and 1 mammal (Table 2.2, Appendixes 1 and 2). Additionally, Appendix 2 presents the categories based in the Red Book of wild Bolivian vertebrates which also includes reptiles (Ministerio de Medio Ambiente y Agua 2009).

Species diversity *vs.* climate

A summary of the localities used in our analyses is presented in Appendix 3. Total plant species richness was significantly and positively correlated with precipitation and length of the growing season (Table 2.3, Fig. 2.5). The opposite relationship was found for elevation. The species richness of shrubs, grasses and trees decreased with elevation. The species richness of grasses was also positively correlated to precipitation and length of the growing season. Fern diversity increased as length of the growing season increased, as did the number of arboreal species. Tree diversity also increased as precipitation increased.

Table 2.2 Conservation status of organisms living in the *Polylepis* woodland remnants according to IUCN (2008, birds and mammals) and Meneses and Beck (2005, for plants), and as function of growth form (plants) and trophic niche (birds and mammals).

		Near threatened	Vulnerable	Endangered & critically endangered	Total
Plants	Herb		1		1
	Shrub		1		1
	Tree		3		3
	Succulent		2		2
	Subtotal	0	7	0	7
Birds	Insectivores	6	1	2	9
	Frugi-granivorous	2		2	4
	Nectarivorous	1			1
	Carnivorous	1			1
	Omnivorous	2	1		3
	Subtotal	10	1	3	14
Mammals	Herbivorous		2		2
	Omnivorous		1		1
	Carnivorous	1			1
	Subtotal	1	3	0	4
	Total	11	11	3	25

Table 2.3 Relationships between the number of species of plants, birds and mammals in relation to elevation and climatic variables (Spearmann rank correlation values). Number of localities in parenthesis, and statistically significant values with asterisk (*P*<0.05).

	Elevation	Tmax	Tmean	Tmin	Prec.	PET[a]	LGS[b]
Plants (n = 31)							
Herbs	-0.10	-0.05	0.02	-0.09	0.31	0.08	0.29
Shrubs	-0.57*	-0.04	0.34	0.35	0.22	0.38*	0.33
Grasses	-0.39*	0.06	0.29	0.21	0.40*	0.30	0.40*
Ferns	-0.25	-0.05	0.03	0.04	0.33	0.09	0.37*
Trees	-0.41*	0.20	0.11	0.20	0.53*	0.17	0.67*
Total	-0.47*	0.15	0.29	0.24	0.51*	0.32	0.54*
Birds (n = 51)							
Insectivorous	-0.12	0.27	0.09	0.15	0.56*	0.11	0.53*
FG[c]	0.05	0.18	0.07	0.06	0.41*	-0.05	0.33*
Nectarivorous	0.09	-0.02	-0.15	-0.01	0.55*	-0.15	0.56*
Carnivorous	-0.02	0.22	0.04	0.13	0.39*	-0.09	0.41*
Total	-0.07	0.22	0.07	0.13	0.53*	0.03	0.51*
Mammals (n = 11)	0.07	-0.65*	-0.34	-0.24	0.47	-0.23	0.51

[a] Potential evapotranspiration
[b] Lenght of the growth season
[c] Frugi-granivorous

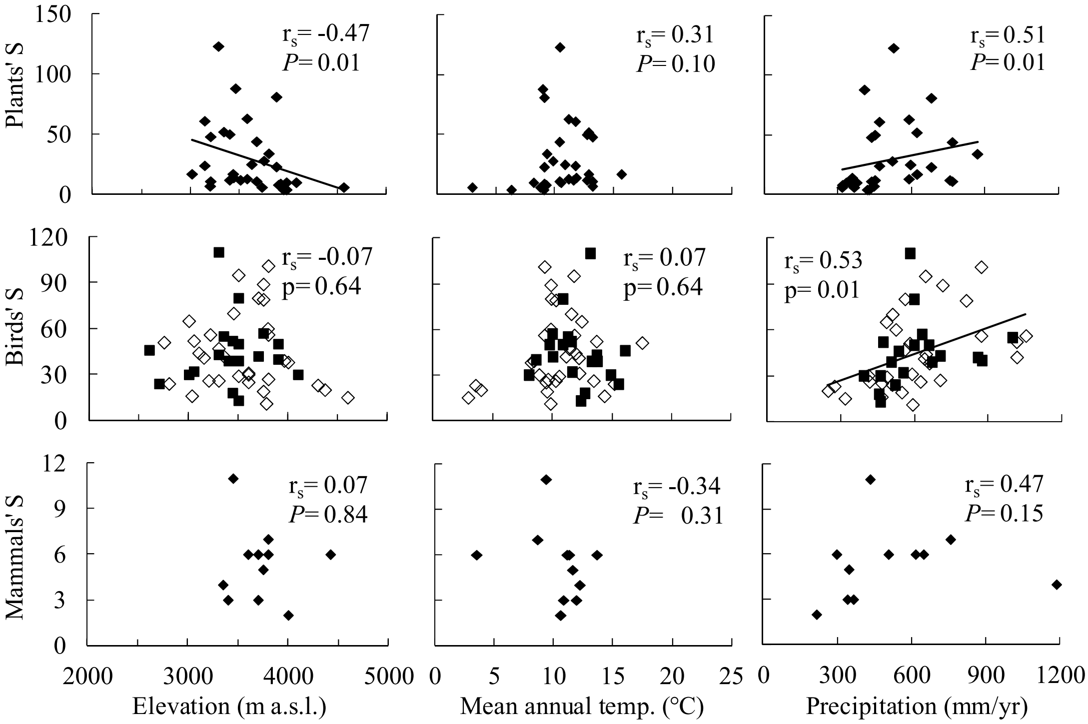

Fig. 2.5 Relationship between elevation, mean annual temperature and precipitation with the total species richness of plants, birds and mammals reported for the *Polylepis* woodland remnants. Spearman correlation values (r_S) and probabilities (*P*) are indicated. For the birds, *white diamonds* represent good quality sites and *black squares* poor quality sites. The *trend lines* in the significant correlations do not mean causality.

Regarding the birds, total species richness (245) positively correlated with total precipitation and length of the growing season (Table 2.3, Fig. 2.5). This trend was found to hold true for insectivorous, frugi-granivorous, nectarivorous as well as carnivorous birds (Table 2.2). The endemism (recorded as the mean inverse range-size for all the species present in a locality) was positively correlated to potential evapotranspiration (r_S=0.29, P= 0.04, Fig. 2.6). When the endemism was calculated only for the 25% most range-restricted bird species (n= 63), the mean endemism score also increased significantly with precipitation (r_S= 0.31, P= 0.03). When introducing habitat quality in the graphs, good and poor quality sites were randomly dispersed in the species richness plots (Fig. 2.5). Habitat quality did not affect bird endemism after correcting for potential evapotranspiration as shown by the covariance analysis (for main and interaction effects $F_{1,47}$=0.01, P>0.9). The endemism plots mostly showed hump-shaped patterns for sites with well matured forest, thus with peak values at intermediate elevations and climates (Fig. 2.6). Concerning the mammals, a negative relationship was found with the maximum temperature, but high (although non significant) values of positive correlation were obtained with precipitation and length of the growth season (Table 2.2). For the other groups, reptiles and butterflies, no test was possible due to the few studies on these organisms.

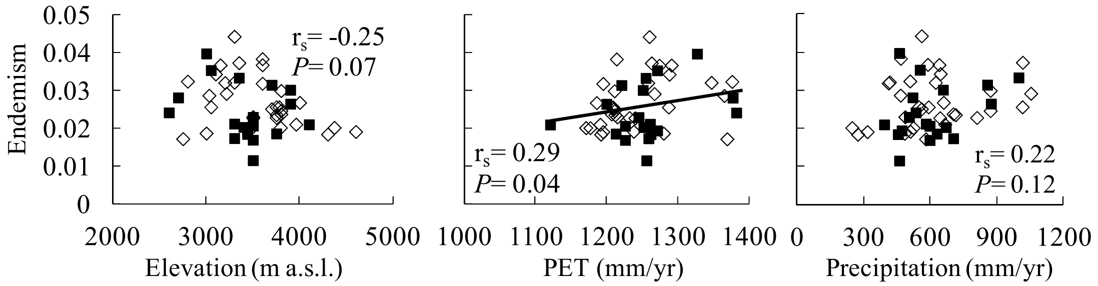

Fig. 2.6 Relationship between elevation, potential evapotranspiration and precipitation with the bird mean endemism reported for the *Polylepis* woodland remnants. Spearman correlation values (r_s) and probabilities (P) are indicated. *White diamonds* represent good quality sites and *black squares* poor quality sites. The *trend lines* in significant correlations do not mean causality.

Discussion

Although it is possible to find structurally similar habitats in mountains of the temperate areas and the tropics, diversity patterns on elevation gradients are different on high and low latitudes, first of all because the amplitude of seasonal change greatly overrides the altitudinal variation at high latitudes (Ghalambor *et al.* 2006). For this reason, the comparisons below are restricted to tropical mountains with emphasis in the Neotropical region.

Cumulative species richness

All surveys of bryophytes were in the vicinity of Cochabamba, in the center of Bolivia, but this is certainly not representative for the diversity of bryophytes of the most humid *Polylepis* habitats of the Yungas province. Surveys of vascular plant species richness covered all the biogeographic provinces in the Bolivian Andes (Fig. 2.1b). However, they did not include woodland remnants of all the *Polylepis* species. This suggests that our estimates of plant species richness are still too low. This is also supported by the cumulative species richness curve, which does not level off yet, suggesting additional plant species can be found in these high mountain ecosystems. The presence of two studies with the same final collection date (*i.e.* 1996; Mercado-Ustariz 1998 and Fernández-Terrazas 1997) explains the steep increase in cumulative species richness observed for plants that year (Fig. 2.3).

Despite these shortcomings, the total plant species richness (537) found in the *Polylepis* woodland remnants is 36% of the species richness estimated for the Bolivian dry Andean valleys at lower elevations than the *Polylepis* woodland remnants (Larrea-Alcázar & López 2005). The maximum species richness per woodland type was 188 vascular plant species in *Polylepis subtusalbida* woodland remnants (Hensen 2002); a value that is higher than the species richness found in the Huaquina sector in Peru (136 spp.; Arteta *et al.* 2006). Concerning the species richness per family, the *Polylepis* woodland remnants contain 17% of the 601 species of Asteraceae reported in Bolivia (Funk *et al.* 2005).

The cumulative bird species richness seems to level off during recent years, indicating that most of the bird species may have been found. Many collecting expeditions in Bolivia early in the 20th century collected birds in *Polylepis* forests, but the habitat is not specified in the publications, so only from the 1970s are bird records for *Polylepis* habitats specifically mentioned (unlike in Peru, where there were several early accounts of the avifauna of this habitat). Most of the publications found for the Bolivian Andes included studies since the 1980s (*e.g.*, Fjeldså 1992; Fjeldså 1993; Fjeldså & Kessler 1996; Fjeldså *et al.* 1999; Andersen *et al.* 1999; Herzog *et al.* 2003; Balderrama 2006). The altitudes included in our study (2600-4600m, Appendix 3) are relatively species poor compared with the lower parts of an altitudinal transect in the Bolivian Yungas (Herzog *et al.* 2005). On the other hand, most of the many species of the lower Yungas slope are widespread, and the avifauna of the *Polylepis* zone is outstanding in terms of the numbers of species with restricted distributions.

The cumulative species richness curve of mammals shows a similar tendency as the birds, which reflects a rather complete sampling.

Growth form of plants and trophic niche of animals

More than half of the species richness of plants in the Bolivian *Polylepis* woodland remnants consists of herbs and shrubs (40% and 15%, respectively). Some of them are considered important components of the climax community (Fernández-Terrazas 1997; Navarro *et al.* 2005), such as the herbs *Azorella compacta* and *Mutisia lanigera*, and the shrubs *Berberis commutata* and *Dasiphyllum hystrix*.

Most of the bird species are insectivorous and frugi-granivorous (together >75%; Fig. 2.4b), which shows the strong dependency of birds on vegetation and insects. It seems in general that there are few phytofagous insects that live from the *Polylepis* trees, but because of the rich associated vegetation (of well matured forest remnants) and the complex structure of *Polylepis* trees, these trees provide important refuge for insects and large numbers of arachnids (Fjeldså & Kessler 1996). However, the plant and insect species diversity still requires much more study. It is known that the insectivore guild of bird core species (those that regularly breed, winter, or migrate through a given habitat, Remsen Jr. 1994) was temporally stable across the year at Sacha Loma, a locality in the Peruvian Puna biogeographic province (Herzog *et al.* 2003), indicating that an adequate supply of insects may be present during the whole year in these high Andean woodland remnants. Despite this, seasonal variation at the community level reflected that of frugi-granivorous and nectarivorous species, which is lowest in June-July, and highest when food resources were available, from October until November (Herzog *et al.* 2003).

As in birds, insectivorous, herbivorous and nectarivorous mammals (altogether > 75%), were the most abundant guilds; suggesting again the key role of insects in these patches of dense vegetation in the high Andes. Also five of the eight reptiles described for *Polylepis* woodland remnants were insectivorous. Thus, insects may be an important part of the *Polylepis* woodland remnants.

Conservation status of the species inhabiting the *Polylepis* woodland remnants

It is noteworthy that most of the birds and mammals living in the *Polylepis* woodland remnants were evaluated by the IUCN as candidates for red list species (IUCN 2008), whereas other groups, such as reptiles and butterflies, have no species evaluated. It is clear that for these, additional research is needed. In total, 3% of the species diversity of the *Polylepis* woodland remnants has some degree of threat: 1.6% of the plants, 5.2% of the birds and 8.7% of the mammals. Most of the organisms have been classified as near threatened (11) and vulnerable (11), which show it is time to act in order to avoid their extinction in the wild (Table 2.2). There are also three endangered species, which have a very high risk of extinction in the wild: two insectivorous birds (*Anairetes alpinus*, *Cinclodes aricomae*) and one frugi-granivorous bird (*Poospiza garleppi*).

Species diversity *vs*. climate

The positive correlation between plant species richness (including trees and grasses) and precipitation may represent a more general pattern for tropical forests (Clinebell *et al.* 1995; Hawkins *et al.* 2003). The negative relationship between total plant species richness (also for the number of shrubs, grasses and trees) and elevation (Table 2.3) was also found for trees in neotropical forests (Gentry 1995); although, plant groups which require high air humidity may show peak at mid-elevation or even higher (Kessler *et al.* 2001; Herzog *et al.* 2005). Even though the declining species richness at high elevation may be mediated by temperature, which decreases with elevation, no significant relationship was found between temperature and plant species richness. It is worth noting here that well matured *Polylepis* woodlands with a closed canopy are mostly frost-free by night even when the grassland right outside the forest edge is frostbitten (Jon Fjeldså, pers. obs.); furthermore, most *Polylepis* woodlands are found on sloping terrain, in places where cold air does not accumulate by night (Fjeldså and Kessler 1996; Kessler 2002). Thus, plant species richness in the *Polylepis* woodlands may be more related to local temperatures instead of being related to the general ones. The positive correlation between length of the growth season and plant species richness (including grasses, ferns and trees) is related to the fact that a longer growth season and a higher amount of water (precipitation) are related with a higher system productivity, which might lead to increased species richness (Begon *et al.* 1996).

For birds, we found a positive correlation between total species richness and precipitation, as well as length of the growth season. The same relationships were also significant for all the analyzed guilds and might be linked to a higher plant species richness which is found with higher productivity conditions. The higher plant species richness may offer more resources and niches for birds, increasing the bird species richness. However, this increase is mainly due to widespread species responding mostly to water and energy, and not to the range-restricted ones, whose distributions are better explained by topography and landscape complexity (Rahbek *et al.* 2007), as well as historical persistence of populations in stable places (Fjeldså *et al.* 1999; Fjeldså & Irestedt 2009). Concerning the relationship between bird species richness and elevation, a monotonic decline in the species richness with increasing elevation was once considered a general pattern for birds and mammals (Begon *et al.* 1996), but it seems now that a hump-shaped pattern may indeed be more typical (Rahbek 1995; Lomolino 2001; Herzog *et al.* 2005). The peak diversity for Andean birds is typically at the lower slopes of the Andean mountain range (*c.* 500m a.s.l., Fjeldså & Irestedt 2009), there is a decrease at intermediate elevations (1000-1750m, Herzog *et al.* 2005) that may continue until 4000m (Fjeldså & Irestedt 2009) or may have a plateau (1750-3250m, Herzog *et al.* 2005). Finally, there may be local peaks in the montane basins with intermediate humidity, as indicated in Fig. 2.5 (3300-3800m). A full understanding of these patterns may require more fine-grained climate data, which detects the local variation such as position of moisture-retaining mist zones and patterns of movements of cold air on the transition between the mountain basins and the high plains.

The distribution of poor quality sites in the plots of species richness (Fig. 2.5) may reflect the fact that site quality is not much related to species richness under an intermediate disturbance hypothesis (Connell 1978), as the spatiotemporal heterogeneity of habitats can lead to high turnover and species richness. The highest endemism was observed at intermediate elevations, temperatures and precipitation. Thus, maximum levels for well-matured habitat patches was at 600mm, which generally corresponds to elevations above 3000m in the rain-shadow basins (*e.g.* in the endemism center of Cochabamba, see Fjeldså 2002). Fig. 2.6 suggests high values also in some of the wettest forests, but often the specialized *Polylepis* species are displaced, maybe by competition, by widespread cloud-forest birds from *Polylepis* patches in the humid zone.

Concerning the mammal species richness, we found a significant negative correlation with the maximum average monthly temperature, and high correlation values with the length of the growing season and precipitation. The negative correlation with maximum temperature may be related to an increase in water loss that may occur in small mammals under hot conditions (Cortes *et al.* 2000). Yensen and Tarifa (2002) found a positive relationship with the precipitation, see Fig. 2.5 (despite two outliers, one at high precipitation values and the other at high species richness values). The expected pattern of declining species richness with increasing elevation (Begon *et al.* 1996) was not observed in our study. This pattern may be clearer if studies from a broader elevational range were done.

Knowledge gaps and conclusions

The increasing number of publications regarding *Polylepis* in general, and published in Bolivia, in particular, is promising and shows the increasing interest for these threatened woodland remnants. However, more research is still needed. None of the studies analyzed surveyed plant species richness for the woodland remnants with *P. crista-galli*, *P. hieronymi*, *P. incarum*, *P. pacensis*, *P. pauta*, *P. pepei, P. sericea*, or *P. triacontandra*.

Most of the studies for the different groups were conducted in the Cochabamba region, one of the key areas for the conservation of the *Polylepis* woodland remnant avifauna (Fjeldså 2002). Therefore, more research is needed in other regions with *Polylepis* woodland remnants. Comparing the *Polylepis* distribution described by Fjeldså and Kessler (1996) with the distribution of the study sites used in this paper, we suggest the following zones as areas for additional field surveys: 1) for plants (bryophytes and vascular plants), the centre and west of the Peruvian-Bolivian Yungas, the west and centre of the Peruvian Puna provinces, and the south of the Bolivian-Tucuman and Altiplanic provinces. Although the accumulation curves suggest that most of the bird and mammal species have been found in the sampled areas, additional efforts are required: 2) for birds, in the west of the Peruvian-Bolivian Yungas province, in the west of the Bolivian-Tucuman province, and the south of the Altiplanic province; 3) for mammals in the south of the Altiplanic biogeographic province, the west of the Bolivian-Tucuman, and the west and centre of the Peruvian Puna and the centre of the Yungas region (Fig. 2.1d). Regarding the reptiles, insects and Fungi, only one study has been found per group. Obviously more research is needed for these groups. Although clear gaps in knowledge do exist, and more species will be found in these woodland remnants, the total number of species observed in these 31 studies amounts to 780 identified species.

Clearly, *Polylepis* woodlands can be considered high altitude biodiversity islands in Bolivia. So far, most research on *Polylepis* forests in Bolivia was very descriptive, reporting occurring species. Much less research has dealt with the processes threatening the occurring species in Bolivia. Most of the *Polylepis* species themselves in Bolivia are threatened to some extent, and habitat fragmentation, burning, overgrazing, soil erosion and firewood collection have been mentioned as the main processes (IUCN 2008). Studies regarding the effect of burning, grazing and soil erosion were mainly performed in Argentina and Ecuador (Renison *et al.* 2002; Renison *et al.* 2004; Teich *et al.* 2005; García *et al.* 2008; Torres *et al.* 2008; Cierjacks *et al.*

2008a; Cierjacks *et al.* 2008b), and only a few studies addressed these issues in Bolivia (*e.g.*, Kessler & Herzog 1998; Hensen 2002). Also the effects of global change on *Polylepis* forest are largely understudied and we found only one study on this issue in Argentina (Marcora *et al.* 2008). Although field surveys remain required in order to acquire complete accounts of species diversity in *Polylepis* woodlands, much more efforts are necessary to get insight in the processes threatening their exceptional biodiversity.

CHAPTER 3

Germination ecology of *Polylepis besseri*

Gareca, E.E., F. Vandelook, M. Fernández, M. Hermy, & O. Honnay. Seed germination, hydrothermal time models and the effects of global warming on a threatened high Andean tree species. *Seed Science Research* (accepted).

Introduction

Timing of germination and seedling emergence are crucial in the life cycle of plants (Harper 1977). They influence posterior plant fitness components such as survival, growth and fecundity in different ways (Verdú & Traveset 2005), and in perennial species the benefits acquired by germinating and emerging early are perpetuated for many years after germination (*e.g.* De Luis *et al.* 2008). Germination is the first major developmental transition in the life of plants affecting the evolution of post-germination traits, ecological niches and geographic ranges of plants (Donohue *et al.* 2010). In short, a plant bets its life in this all or nothing event (Welbaum *et al.* 1998).

Climate change may have very important consequences for the population and community dynamics of plant species through their effects on sexual plant reproduction (Hedhly *et al.* 2009; Walck *et al.* 2011). The immediate effects of global warming have been shown to be mediated by early flowering (Menzel *et al.* 2006) and an increase in or irregular seed production (Porter 2005; Barnabás *et al.* 2008). Climate change effects on seed germination have been examined by means of observational studies (Dainese 2011) and common garden experiments (Willis & Hulme 2002; De

Frenne *et al.* 2010), and by modifying field temperatures (Zavaleta 2006; Klady *et al.* 2011), CO_2 concentration (Edwards *et al.* 2001; Zavaleta 2006), and water availability (Cipriotti *et al.* 2008; Lampei & Tielbörger 2010), both in greenhouse (de Dios Miranda *et al.* 2009) and in laboratory experiments (Graae *et al.* 2008; McCarragher *et al.* 2011). However, most of these have not modelled germination under the whole range of conditions a species may experience in the future.

Temperature and water availability are important regulators of seed germination (Kebreab & Murdoch 1999; Bradford 2002). Threshold models have been developed to understand how both factors affect the germination process. More specific, hydrothermal time (HTT) models (Gummerson 1986; Bradford 1990; Alvarado & Bradford 2002) indicate that the timing of germination is closely related to temperature and water potential thresholds for radicle emergence which vary among individual seeds in a population (Welbaum *et al*. 1998). HTT models describe seed germination using parameters such as base, optimum and ceiling germination temperatures, hydrothermal time required for germination, and mean and standard deviation of the base water potential for the seed population (Dorado *et al.* 2009; Meyer & Allen 2009). The latter is the threshold water potential for radicle emergence of a given seed or seed fraction (Bradford 2002). Once these parameters are estimated, germination time courses can be predicted for any combination of temperature and water potential (Allen 2003). So far, HTT models have been developed mainly in an agronomical context to predict emergence of crop and weed species (*e.g.* Roman *et al.* 1999; Alvarado & Bradford 2005). They showed to be effective in disentangling the effect of pre-germination treatments (Cheng & Bradford 1999; Alvarado & Bradford 2005), predicting dormancy loss and field germination (Meyer & Allen 2009), explaining differences in germination patterns associated with contrasting habitats (Allen *et al.* 2000), seed sizes (Wang *et al.* 2004), and genotypes (Ellis *et al.* 1986; Dahal *et al.* 1990). Because HTT models can predict how any specific fraction of a seed population will respond to alterations in environmental conditions (Bradford 2002), these models can also be applied to study the response of rare and threatened plant species to climate change (Wei *et al.* 2009; Qiu *et* *al.* 2010). Although HTT models were mainly used in species with short lifespan, they are also specifically relevant for species with long generation times, for which adaptation in response to a rapidly changing climate needs a century or over a millennium to occur (Davis *et al.* 2005).

Species from mountain ecosystems, and particularly of mountain summits, are very susceptible to climate change given their inability to keep up with the upward range shift and the accompanying habitat reduction (Fischlin *et al.* 2007). One particular genus finding itself in this situation is *Polylepis* (Rosaceae). Knowledge on germination requirements or possible dormancy mechanisms in *Polylepis* species is scarce. In general little information is available on the germination ecology of tropical mountain plants. The seed germination of tropical high mountain species is mainly determined by light, temperature and water availability (Teketay 2005). It was found that the germination of five species of the sister genus *Acaena* (Rosaceae) from the subalpine zone in New Zealand (not in the tropics) had an optimum temperature of 20°C, except for *A. glabra* (17°C) (Baskin & Baskin 1998; Conner 1987). Germination of these species was promoted by light, increased at high water potentials and had a narrow temperature range for germination (14-21°C); cold storage or alternating temperatures did not affect germination, which was higher under light conditions compared to darkness (Conner 1987). As far as we know, there is no information about the germination ecology of *P. besseri* (one of the important *Polylepis* species in the Peruvian Puna biogeographic province in Bolivia, see Chapter 1) and as for the other *Polylepis* species in Bolivia, its populations have an uncertain fate under current climate change (Gareca *et al.* 2010b, see Chapter 2).

Our general objectives were to apply HTT models to study the germination characteristics of *P. besseri* and its response to changes in water availability and temperature conditions. The specific objectives were to: 1) check for possible dormancy mechanisms; 2) construct a hydrothermal time model for the species and derive important germination thresholds and parameters; 3) examine whether fluctuating temperatures stimulate germination; and 4) based on the parameters obtained from the HTT model, discuss effects of climate change, and specifically of increasing temperature, on the germination of *P. besseri*.

Materials and methods

Study species

Polylepis besseri Hieron. is a tree distributed at 3000-4100m a.s.l. in Cochabamba and Chuquisaca – Bolivia, and grows about 8 m tall (Fjeldså & Kessler 1996). *P. besseri* woodlands in Sacha Loma and Cuturi (17°44'S, 65°34'W, Cochabamba) are among the largest and most dense *Polylepis* woodlands left within a 60km range from the city of Cochabamba (Cahill & Matthysen 2007). At this site, *P. besseri* flowers between July and December with a peak in September and October, and produces fruits between September and April with a peak in December (Martinez-Costas 2003), when most fruits are mature (Edgar E. Gareca, pers. obs.). Each fruit (the dispersal unit) is typically a one-seeded achene (Simpson 1979). Seeds germinate and seedlings emerge throughout the warm rainy season from January to April (unpublished data). The range of maximum, mean and minimum temperatures during this season are 12.7 to 18.4°C (during the day), 7.6 to 11.4°C, and -1.1 to 2.7°C (during the night), respectively (values retrieved from the Local Climate Estimator New Loc Clim v. 1.10 using the Shepards method with a vertical and horizontal correction (*c.f.* Grieser *et al.* 2006)).

Plant material

Fruits of *Polylepis besseri* were collected between December 2009 and January 2010. Mature fruits were manually collected from at least 50 trees in each of four large fragments found near Sacha Loma at elevations between 3600m and 3810m a.s.l.. The plant material was stored at room temperature (*c.* 20°C) for one week inside newspapers allowing it to dry. Afterwards, fruits were cleaned (leaves and small branches removed) and healthy fruits (*i.e.* complete fruits without holes) were selected for, mixed and sent to Belgium, where they arrived two weeks later and immediately used for germination tests.

Fruit coat permeability and water imbibition

Permeability of the fruit coat for water was determined by means of scarification whereby part of the fruit coat was chipped off with a scalpel. The experiment included two treatments, scarifying and non-scarifying fruits, with three replicates of 25 fruits each, making a total of 150 fruits. The fruits were incubated in germination chambers at 20°C during the day and 2°C during the night (12h day and 12h night) in Petri dishes with moist filter paper (MN 440). Light was provided by fluorescent tubes (Philips TLD 80) with a photon flux density of 52 µmol/(m^2·s), 400–700 nm. The weight of the 25 blotted fruits per Petri dish (replicate) was measured at the beginning of the experiment, every hour for 6h, every 6h for 18h, every 24h for 2d, and every 72h for 6d, making a total of 216h of experiment. This evaluation timing was taken because of the known water uptake pattern of the seeds with three phases: first, it has a rapid initial water uptake (*i.e.* imbibition), followed by a plateau phase and a final water uptake phase as the embryo axis elongates and breaks the covering layers to complete germination (Finch-Savage & Leubner-Metzger 2006). The increase in weight of the 25 fruits was estimated at each evaluation event as the difference of the observed and initial weight divided by the initial weight of the fruits (Baskin & Baskin 1998).

Hydrothermal time model

The experiment included a full factorial design of treatments coming from two factors: water potential and temperature. The water potential levels were 0.0, -0.3, -0.6 and -0.9MPa and represent decreasing levels of water availability for seed germination; and the constant temperatures used were 5, 9, 15, 20, 25, and 30°C, making a total of 24 treatments. This range of temperatures covers the temperatures that seeds experience in nature during the germination period (5-15°C) and higher values to check for the effect global warming (20-30°C) on germination. The water potential levels were prepared with Polyethylene Glycol 8000 (PEG; Biochemica, Germany) according to Michel (1983), using different concentrations for different temperatures. Each treatment had three replicates; each replicate consisted of 10 Petri dishes with 25 fruits each, making a total of 250 fruits per replicate. This amount of fruits per replicate was used to account for the low and variable germination percentages reported for related species: *Polylepis australis* (Renison & Cingolani 1998; Enrico *et al.* 2004; Marcora *et al.* 2008), *P. incana* (Driesch & Kessler 1996) and *P. subtusalbida* (Gareca *et al.* 2007). Eight millilitres of the corresponding PEG solution was added to each Petri dish and it was sealed with parafilm to reduce evaporation. The solutions were renewed two times during the experiment, after the 5th and 10thwk of experiment, to avoid fluctuations in water potential. Germination, which finishes with the visible emergence of embryonic tissues from the seed or from the fruit in this case, was evaluated every week until no new germinations were detected for two consecutive weeks in most of the treatments (16wk). The number of white firm seeds present in the fruits was evaluated at the end of the experiment by cutting fruits in half. The total number of seeds germinated was divided by the total number of available seeds to obtain the percentage of germinated seeds. Germination percentages were averaged over the three replicates before continuing with the analysis.

To determine the optimum germination temperature (*i.e.* the temperature where germination rate is at its maximum) a plot of germination rate *vs.* temperature was drawn (Fig. 3.1). Least square regression lines were fit to the 10th and 20th percentile above and below the visually estimated optimum temperature (T_o), which was between 20 and 25°C. Optimum temperature per percentile was estimated as the point where regression lines above and below the visually estimated T_o crossed each other.

The germination modelling was based on the HTT models (Gummerson 1986; Bradford 1990; Alvarado & Bradford 2002) which assume that the base temperature (T_b) and the hydrothermal time required for germination (θ_{HT}) are constants, while the base water potential (ψ_b) varies according to a normal distribution and is characterized by its mean ($\psi_{b(50)}$) and standard deviation ($\sigma_{\psi b}$; Gummerson 1986). The models also assume that below the optimum temperature (T_o) ψ_b is independent from temperature, and that the T_b is independent from water potential (Kebreab & Murdoch 1999). Above T_o, where it is assumed that the hydrothermal time accumulation is maximal, ψ_b increases with increasing temperature with a slope equal to k_T

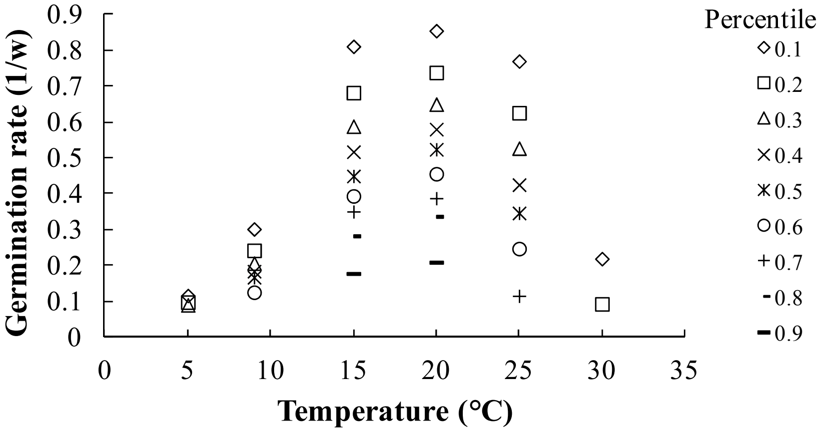

Fig. 3.1 Germination rate of *Polylepis besseri vs.* temperature for each percentile of germination (*symbols*).

(Alvarado and Bradford 2002). The modelling was performed using repeated probit analyses (Ellis *et al.* 1986) where the response variable, percentage of germination, was transformed to the probit scale using the PROBIT function in SAS (SAS Institute Inc. 2008). The probit and logit distributions are essentially identical (Bradford 1995). All the theory and practice of probit models can be rewritten in terms of this alternative transformation, and extremely large experiments would be needed to show one has a better fit than the other for a particular dataset (Finney 1971). In fact, it has been shown that for practical purposes, the results of the two methods are identical (Bradford 1995). We used the probit transformation here because most of the research on HTT modelling was developed using it (*e.g.* Gummerson 1986; Bradford 1990; Alvarado & Bradford 2002). Values below 5% and above 95% of the final germination were considered data points that did not add any germination, as well as any observation where no increase in germination percentage occurred, and therefore excluded; as a consequence 158 observations out of 1152 initial observations were used in the analysis. A simple linear regression of probits *vs*. the respective predictor variable per model was performed using PROC REG in SAS for each model (Table 3.1; SAS Institute Inc. 2008). We changed different parameters repeatedly in the predictor variable until the highest R^2 was obtained for the simple linear regression, except for the hydrothermal time model below the optimum temperature, where besides maximizing the R^2 we aimed to retrieve similar parameters for the best hydrothermal time model above optimum temperatures. The equations, the predictor variables for obtaining the predicted germination, as well as the methods for obtaining the parameters are summarized in Table 3.1. Goodness of fit of the models was checked by constructing plots of germination percentage *vs*. the normalized thermal time (Bradford 2002; Alvarado & Bradford 2002).

Fluctuating temperatures

Fruits were incubated in three treatments: a constant temperature of 20°C, and the daily fluctuating temperatures 20/2°C and 20/10°C (12h day/12h night), with three replicates. The 20/2°C treatment resembles the large daily temperature fluctuations in the study area and the 20/10°C treatment has been shown to stimulate germination of many species (Thompson *et al.* 1977; Thompson & Grime 1983). Germination was evaluated as in the hydrothermal time experiment during 19wk.

Finally, the mean germination rate was calculated as the inverse of the time to reach 50% of germination. The mean germination rate and the final percentage of germination (arcsin transformed) were compared between fluctuating temperature treatments using a one way ANOVA with three levels of the factor temperature (20°C, 20/10°C and 20/2°C). The normality assumption was checked before the analysis. In case of a significant effect of temperature, one degree of freedom contrasts were estimated between the constant and fluctuating temperatures.

Table 3.1 Equations, their sources, predictor variables, and how the parameters were estimated for each germination model of *Polylepis besseri*.

Model[a]	Equation[a] Probit (g) =	Source	Predictor variable[a]	Parameters obtained by:	
				Changing values	Formula[b]
Thermal time below T_o	$\{\log[(T-T_b)\cdot t_g]-\log \theta_{T(50)}\}/\sigma_{\theta T}$	(Dahal *et al.* 1990) eq. i	$\log[(T-T_b)\cdot t_g]$	T_b	$\sigma_{\theta T} = 1/b$; $\theta_{T(50)} = 10^{(-a/b)}$
Thermal time above T_o	$[T+(\theta_C/t_g)-T_{c(50)}]/\sigma_{Tc}$	Modified after Ellis *et al.* (1986) eq. 8	$T+(\theta_C/t_g)$	θ_C	$\sigma_{Tc} = 1/-b$; $T_{c(50)} = -a/b$
Hydrotime	$[\psi-(\theta_H/t_g)-\psi_{b(50)}]/\sigma_{\psi b}$	(Bradford 1990) eq. 7	$\psi-(\theta_H/t_g)$	θ_H	$\sigma_{\psi b} = 1/b$; $\psi_{b(50)} = -a/b$
Hydrothermal time below T_o	$(\psi-\{\theta_{HT}/[(T-T_b)\cdot t_g]\}-\psi_{b(50)})/\sigma_{\psi b}$	(Dahal & Bradford 1994) eq. 5	$\psi-[\theta_{HT}/\{(T-T_b)\cdot t_g\}]$	θ_{HT}, T_b	$\sigma_{\psi b} = 1/b$; $\psi_{b(50)} = -a/b$
Hydrothermal time above T_o	$\{[(\psi-k_T\cdot(T-T_o)) - \theta_{HT}/((T-T_b)\cdot t_g)] - \psi_{b(50)}\}/\sigma_{\psi b}$	(Bradford 2002) eq. 12	$[(\psi-k_T\cdot(T-T_o)) - \theta_{HT}/((T-T_b)\cdot t_g)]$	T_o, θ_H, k_T	$\sigma_{\psi b} = 1/b$; $\psi_{b(50)} = -a/b$

[a] T_o = optimum temperature for germination; T = temperature in the experiment; T_b = base temperature; t_g = time to germination of the g fraction; $\theta_{T(50)}$ = mean thermal time; $\sigma_{\theta T}$ = standard deviation of θ_T; θ_C = thermal time constant at supra-optimal temperatures; $T_{c(50)}$ = mean ceiling temperature; σ_{Tc} = standard deviation of the ceiling temperatures; ψ = water potential of the experiment; θ_H = hydrotime constant; $\psi_{b(50)}$ = mean base water potential; $\sigma_{\psi b}$ = standard deviation of the base water potential; θ_{HT} = hydrothermal time constant; k_T = slope of the relationship between $\psi_{b(g)}$ and T in the supra-optimal range of T.

[b] a and b are the intercept and slope of the regression functions, respectively.

Predicting germination

Based on the germination models constructed, the germination percentages obtained in the constant and fluctuating temperature conditions were plotted on a normalized thermal time scale. Since the minimum temperature was higher than 0°C (*i.e.* 2°C), it was assumed that thermal time accumulation ceased below the base temperature and resumed when seeds were exposed to warmer temperatures (Ellis & Barret 1994). If the time courses coincide, then the model accounts for the observed germination patterns (Bradford 2002). The expected increase of temperature for the central Andes by 2080-2099 ranges between 1.7 and 4.6°C during the germination period (December to May, Christensen *et al.* 2007). In order to model the germination within these values we estimated germination percentages under constant temperatures after 14wk, in the range between -5 and +5 the average temperature during the germination period (9.5°C) in steps of 1°C.

Results

We found that >90% of viable *P. besseri* seeds germinated under appropriate laboratory conditions. In preliminary tests, warm (20°C) and cold (5°C) stratification, and wet (moist filter paper) and dry (silica gel chamber) regimes did not stimulate germination.

Fruit coat permeability and water imbibition

The increase in weight of scarified and non-scarified fruits was very similar at the end of the experiment (Fig. 3.2). About 156 and 134% of the gain in weight was attained during the first 24h of the experiment in the scarified and non-scarified fruits, respectively. This difference disappeared completely towards the end of the experiment (9d) as fruits in both treatments ended with a 210 and 212% increase in weight, respectively.

Germination modelling

In total, 80.5% of the *P. besseri* fruits were empty, or contained gray or underdeveloped seeds. The results provided here are based only on the viable 19.5% of seeds. The estimated optimum temperature (T_o) for *P. besseri* germination was 22.3°C for both, the 10th and 20th percentile. The thermal time model for the suboptimal temperatures at -0.9MPa explained only a small amount of the variation in germination (R^2=0.24; Table 3.2A). The models at higher water potential performed much better as they explained more than 78% of the variation. The germination curves under normalized thermal time (*i.e.* a time scale with the amount of °C above the base temperature for germination times the time to reach a given germination percentage) for the below and above optimum temperature showed that the models accounted for the observed germination patterns, as indicated by the overlap of the different data points (Figs. 3.3 and 3.4).

Regarding the parameters obtained from the thermal time model of germination below T_o, the base temperature (T_b) ranged from 0 to 3.7°C and the thermal time to reach 50% of the germination ($\theta_{T(50)}$) increased exponentially as water potential decreased (Table 3.2A), with the shortest $\theta_{T(50)}$ equal to 33.5°C·w in pure water (0.0Mpa). Above T_o, the thermal time model (Table 3.2B) predicted that the ceiling temperature for 50% of the seeds ($T_{c(50)}$) was 27.9°C, with a very similar value for water availability equal to ψ = -0.3MPa (27.5°C). The values of the thermal time above optimum temperature (θ_C) increased with decreasing water potentials.

The amount of hydrotime required to germinate (θ_H) decreased with increasing temperature up to 25°C (Table 3.2C). Starting from 15°C, the mean base water potential ($\psi_{b(50)}$) increased with increasing temperatures. The standard deviation of the base water potential ($\sigma_{\psi b}$) in the hydrotime model decreased with increasing temperature until 20°C, above which it increased again (Table 3.2C). The increase in $\psi_{b(50)}$ was more pronounced at high temperatures (25 and 30°C) than at low temperatures (5-20°C). The mean base water potential was different for each temperature below the optimum temperature, and no clear trend was observed.

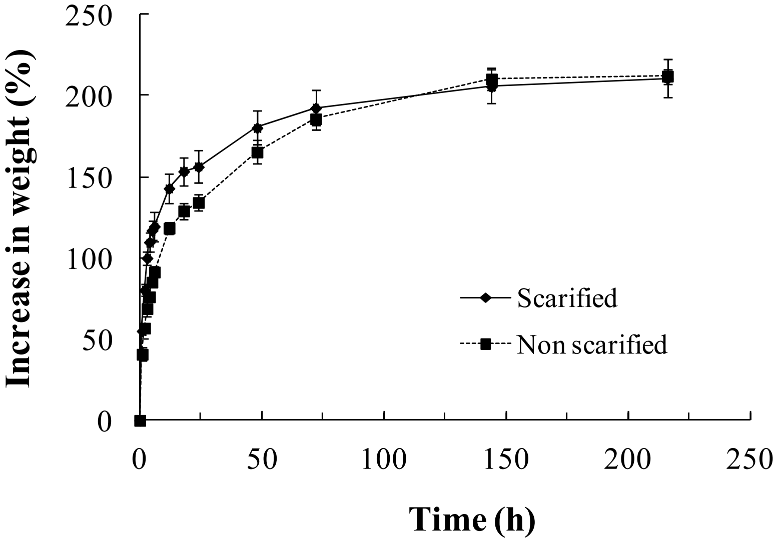

Fig. 3.2 Increase in weight (%) as function of time due to the water uptake of scarified (*diamonds*) and non scarified fruits (*squares*) of *Polylepis besseri.* The *bars* represent standard errors.

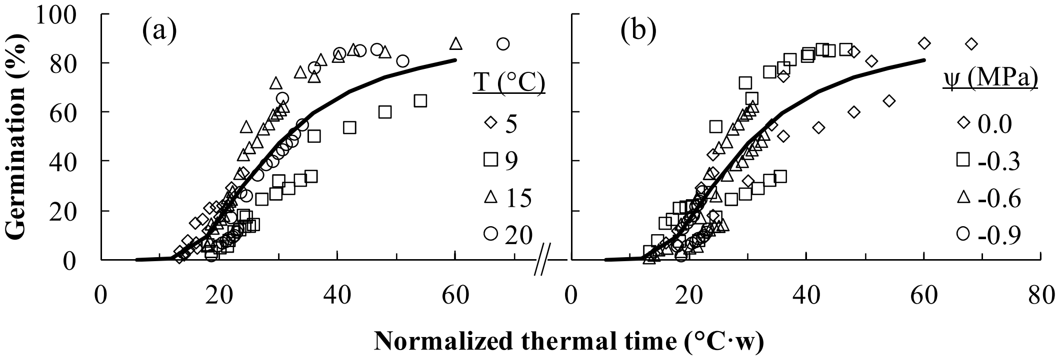

Fig. 3.3 Germination profiles of *Polylepis besseri* at (A) suboptimal temperatures (5, 9, 15 and 20°C) and (B) different water potentials (0.0, -0.3, -0.6, and -0.9MPa) on a normalized thermal time scale. The *symbols* are the actual data and the *lines* are the time courses predicted by the hydrothermal time model using the parameters in Table 3.2D.

Table 3.2 Parameters of the germination models for *Polylepis besseri* using the average of the three replicates per treatment for an optimum temperature between 20 and 25°C.

A	T (°C)	ψ (MPa)	T_b (°C)	$\theta_{T(50)}$ (log°C·w)	$\sigma_{\theta T}$ (log °C·w)	$\theta_{T(50)}$ (°C·w)	R^2
	5-20	0.0	3.1	1.52	0.24	33.46	0.90
	5-20	-0.3	2.9	1.71	0.29	51.74	0.79
	5-20	-0.6	3.7	2.12	0.47	133.19	0.82
	5-20	-0.9	0	3.19	0.89	1560.52	0.24

B	T (°C)	ψ (MPa)	$T_{c(50)}$ (°C)	θ_C (°C·w)	σ_{Tc} (°C)	R^2
	25-30	0.0	27.9	9	3.3	0.99
	25-30	-0.3	27.5	14	3.6	0.93
	25-30	-0.6	24.6	14	3.6	0.98
	25-30	-0.9	---	---	---	---

C	T (°C)	ψ (MPa)	θ_H (MPa·w)	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	R^2
	5	-0.9-0	10.7	-0.74	0.45	0.88
	9	-0.9-0	3.1	-0.47	0.35	0.99
	15	-0.9-0	1.8	-0.84	0.31	0.97
	20	-0.9-0	1.2	-0.71	0.26	0.96
	25	-0.9-0	1.2	-0.45	0.40	0.94
	30	-0.9-0	2.7	0.17	0.60	0.83

D	T (°C)	ψ (MPa)	θ_{HT} (MPa·°C·w)	T_b (°C)	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	R^2
	5-20	-0.9-0	23.0	3.0	-0.74	0.41	0.78

E	T (°C)	ψ (MPa)	k_T (MPa/°C)	T_o (°C)	θ_{HT} (MPa·°C·w)	T_b (°C)	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	R^2
	25-30	-0.9-0	0.1	21.7	23.0	3.0	-0.74	0.41	0.89

(A) Thermal time for suboptimal temperatures at each water potential. (B) Thermal time model for supra-optimal temperatures at each water potential. (C) Hydrotime model for each temperature. (D) Hydrothermal time model for suboptimal temperatures. (E) Hydrothermal time model for the supra-optimal temperatures. See Table 3.1 for a description of the parameters.

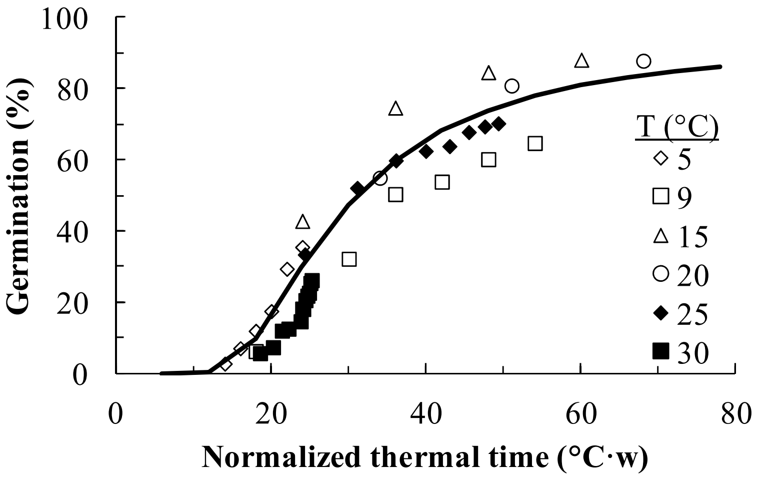

Fig. 3.4 Germination profiles of *Polylepis besseri* at suboptimal (5, 9, 15 and 20°C) and supra-optimal temperatures (25 and 30°C) in pure water (ψ=0.0MPa) on a normalized thermal time scale. The *symbols* are the actual data and the *line* is the time course predicted by the hydrothermal time model using the parameters in Table 3.2E.

The estimated base temperature in the HTT model below T_o was 3.0°C and the water potential threshold preventing radicle emergence for 50% of the germinated seeds ($\psi_{b(50)}$) was -0.74MPa with a standard deviation ($\sigma_{\psi b}$) of 0.41MPa (Table 3.2D). Finally, the HTT model above T_o estimated a T_o of 21.7°C, an increase of the $\psi_{b(50)}$ with 0.1MPa per degree centigrade of increase in temperature (k_T), and a constant θ_{HT} of 23.7MPa·°C·w.

Fluctuating temperatures

Fluctuating temperatures had a significant effect on the rate ($F_{2,6}$= 216.0, P< 0.01) and final germination percentage ($F_{2,6}$= 28.5, P< 0.01) of *P. besseri* fruits (Fig. 3.5). The final germination was similar between 20°C and the fluctuating temperature treatment 20/10°C (P= 0.24) with a common mean of 93% of germination, but it was significantly different for the 20/2°C treatment (P= 0.01) with a final germination of 71%. The time to reach 50% of germination was different in all treatments (P= 0.01 for both comparisons between the constant and fluctuating temperatures), with 1.8wk for the fastest treatment (20°C), 2.7wk for 20/10°C and 5wk for the slowest treatment (20/2°C). Finally, fluctuating temperatures did not stimulate germination; on the contrary, they resulted in less germination in the 20/2°C treatment.

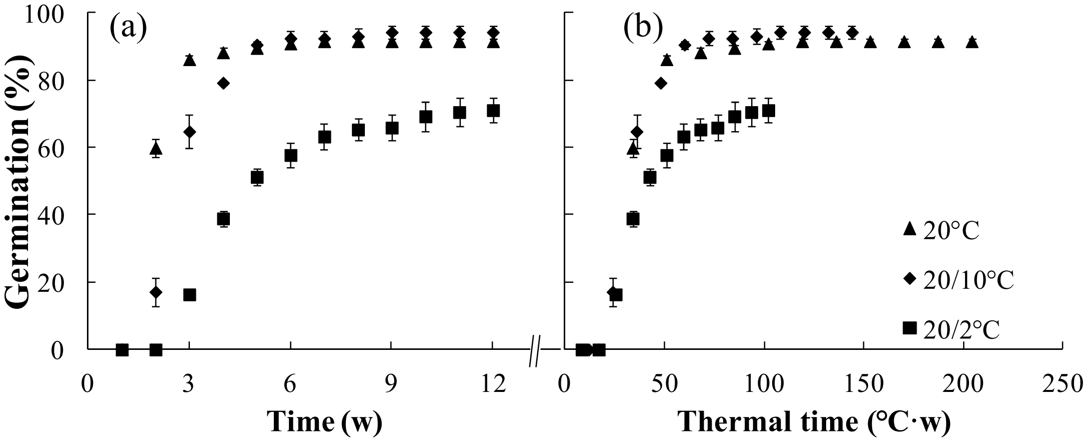

Fig. 3.5 Observed percentages of germination for *Polylepis besseri* at constant (20°C, *triangles*) and fluctuating temperatures (20/10°C *diamonds*, and 20/2°C *squares*) on a (a) time and (b) thermal time scale using the parameters in Table 3.2D. The *bars* represent standard errors.

Predicting germination

The hydrothermal time model developed for the germination of *P. besseri* at constant temperatures also performed well in predicting germination at the fluctuating 20/10°C treatment (Fig. 3.5). This can be seen by the overlapping curves at 20°C and 20/10°C (Fig. 3.5b), where a given germination percentage occurred after the accumulation of the same amount of thermal time, something that did not occur under a regular time scale (Fig. 3.5a). However, the model performed poorly to predict germination in the fluctuating 20/2°C treatment, which included temperatures below the optimum. The HTT model below T_o predicted that increasing temperatures from the field mean temperature (9.5°C) will increase germination under all water availability conditions (Fig. 3.6), and that decreasing temperatures will diminish germination with a more pronounced effect at water potentials close to 0MPa.

Additionally, the HTT models predicted that an increase in temperature near the base temperature for *P. besseri* will increase its germination because temperatures will be above T_b for the species, allowing germination. Whereas temperatures rise above T_o they will reduce *P. besseri* germination because of the increase in base water potential given by k_T (Table 3.2E).

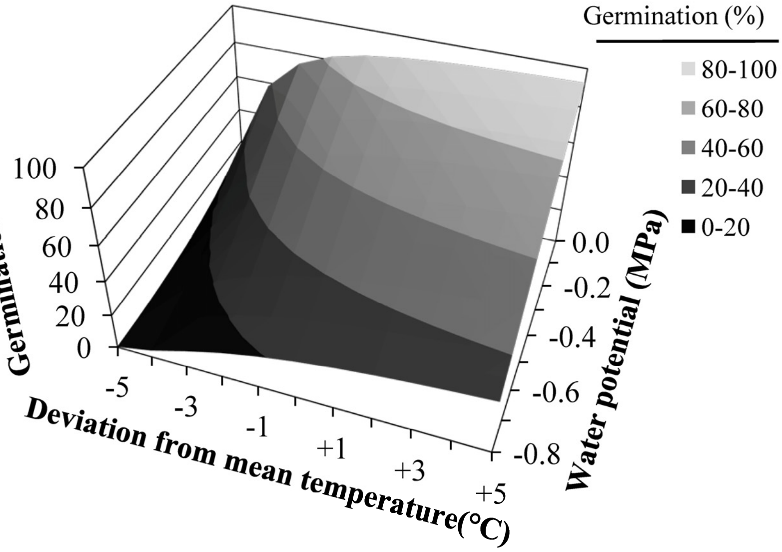

Fig. 3.6. Predicted germination percentages of *Polylepis besseri* under constant temperatures after 14wk, as function of the deviations from the mean average temperature (9.5°C; *c.f.* Grieser *et al.* 2006) and the different water potentials. Different *gray intensities* represent different germination percentages. The predicted values were obtained using the parameters in Table 3.2D.

Discussion

Hydrothermal time models for *Polylepis besseri* could be constructed straightforwardly due to the lack of seed dormancy. The models predicted germination of *P. besseri* under constant and alternating temperatures, except for those temperatures partially below the estimated base temperature (20/2°C). Alternating temperatures did not increase the germination percentage of *Polylepis*; on the contrary, they reduced the germination rate (at 20/10°C) and the final percentage of germination (at 20/2°C). HTT models predicted an initial increase in *P. besseri* germination under global warming and under constant temperatures for the analyzed water potentials. This increase may, however, be countered if temperatures rise too much reaching temperatures above the optimum (21.7°C).

We found that >90% of viable *P. besseri* seeds germinated under appropriate laboratory conditions. Because in preliminary tests warm and cold stratification, and wet and dry regimes did not stimulate germination, we concluded that *P. besseri* produces fruits with seeds ready to germinate. The absence of an effect of cold stratification on the germination was also reported for five species *Acaena* (a sister genus of *Polylepis*) in the subalpine zone in New Zealand (Conner 1987). If, as we propose, *P. besseri* seeds are non-dormant, then germination is the key component of its seedling emergence and increases the importance of modelling germination (Forcella *et al.* 2000).

Modelling

Starting from the base temperature, germination rate increases when temperature rises until the optimum temperature for germination of a given species is attained, then it decreases until a ceiling temperature, above which germination stops (Alvarado & Bradford 2002). Maximum temperatures in Sacha Loma during germination time (12.7 to 18.4°C; Grieser *et al.* 2006) were at least 3.4°C below the optimum temperature for *P. besseri* (21.7°C; Table 3.2E), thus germination is not limited by maximum temperature at this site at the present. The opposite result was found for the base temperature, since the estimates for *P. besseri*'s base temperature (between 2.9 and 3.7°C, except for ψ = -0.9MPa; Table 3.2A) were at least 0.2°C higher than the estimated minimum field temperatures (-1.1 to 2.7°C; *c.f.* Grieser *et al.* 2006). Thus, accumulation of thermal time to germination can be nowadays limited by night temperatures in Sacha Loma. However, it is known that *Polylepis* woodlands create a less cold environment than the surrounding grasslands (Fjeldså & Kessler 1996), thus the effect of night temperatures on germination inside the woodlands may be buffered.

Despite the fact that the hydrotime model detected different mean base water potentials ($\psi_{b(50)}$) and standard deviations ($\sigma_{\psi b}$), the hydrothermal time models, which work with a single value for each of these two parameters and include temperature, explained a good proportion of variability below (R^2= 0.78; Table 3.2D) and above (R^2= 0.89; Table 3.2E) the optimum temperature for *P. besseri*'s germination. The hydrothermal time model (Table 3.2D) predicted that under pure water (ψ= 0.0MPa) and a constant temperature of 21.7°C (the estimated optimum temperature, Table 3.2E), 12, 62, 79 and 85% of germination are reached after 1, 2, 3 and 4wk of experiment, respectively. Maximum germination (94%) occurs after 11wk of experiment and 90% of germination occurs after 6wk. These percentages decrease with decreasing water potential. This information is useful for future germination tests with the species, as 4-6wk of experiment are recommended at this temperature and water potential, a value that is higher than the maximum of 3-4wk recommended for the majority of studies (Baskin & Baskin 1998). Under different conditions, a longer time is needed for the experiments.

A high $\psi_{b(50)}$, which diminishes the germination rate and final percentage of germination (Bradford 1995), and a high hydrothermal time constant, which diminishes germination rate (Bradford 1995), both explain the long time needed for *P. besseri*'s germination under optimal conditions and the high spread of germination over time under low water potentials or temperatures different from the optimum. For other plants, this spread in germination was not expected because early germination provides long-lasting benefits to seedlings because early emergent seedlings are larger, may survive and reproduce more than late emergent seedlings (De Luis *et al.* 2008). However, heterogeneous germination rates can be the result of selection for competition among seedlings after years of high seed production as reported for desert annuals (Tielbörger & Valleriani 2005), because high seedling densities may cause an increase in seedling mortality which in turn may diminish fitness.

Predicting germination

The model developed for constant temperatures could predict the germination at alternating temperatures (20°C in light/10°C in darkness), but not at those including temperatures below the base temperature for *P. besseri* (3.0°C). The seeds at 20/2°C accumulated less thermal time than predicted by the model (Fig. 3.5), or in other words, the model overestimated germination under this condition. Thus, caution must be taken when predicting germination. The model assumed thermal time accumulation ceased below the base temperature and resumed at warmer temperatures, thus no delay in germination was possible when temperatures rose from below to above the base temperature. The overestimation in the model might be caused by a delay in the physiological processes to restart germination after the seeds have been exposed to temperatures below the base temperature. The decrease in the final percentage of germination in the fluctuating 20/2°C treatment in comparison to the 20/10°C or the constant 20°C treatment might be caused by the same delay in physiological processes or it may be evidence of *Polylepis besseri*'s seed damage below the base temperature for germination. The latter is less likely because percentages of germination were estimated based on white firm seeds only, thus potential viable seeds; unless these seeds were damaged enough to avoid seed germination but not to be consumed by fungi and therefore get rotten through the experiment.

Before predicting germination based on the hydrothermal times, it is important to note that actual germination largely depends on prevailing field temperatures, which vary considerably in the high Andes, and much less on averages, which were used for obtaining the hydrothermal time model parameters. However, given (i) that thermal history has no effect on germination rate (Ellis & Barret 1994), which is true for *P. besseri* above the base temperature, (ii) that germination rate responds instantaneously to current temperature (Ellis & Barret 1994), and (iii) analyzing two cardinal temperatures obtained for the germination of *P. besseri* (base and optimum temperatures) we can make some predictions. An increase of 0.2°C in the current minimum field temperature may already release *P. besseri* germination from the limit imposed by its base temperature. On the other extreme, an increase of 3.4°C in the current maximum field temperature, likely to occur in the Central Andes by the end of the century (period 2080-2099; Christensen *et al.* 2007), may start limiting germination because the optimum temperature would be surpassed by the field temperature. In the intermediate condition, *P. besseri* germination will increase by global warming. Regarding the water potential it is expected that, if the precipitation regime remains constant through time, global warming will reduce water availability in the soils, and then it may limit germination (Fig. 3.6).

Thus, general germination pattern predictions through time can be made based on the HTT models: Global warming will first increase *P. besseri* germination because minimum temperatures will no longer limit its germination. Then, germination will be the result of the balance between increased temperatures and reduced water potential. Finally, when maximum temperatures surpass the optimum temperature for *P. besseri*, germination will start to decrease. Since only the maximum estimates for field temperature increase predicted for the end of this century will negatively affect germination, global warming may not be an important limiting factor for *P. besseri* germination in the high Andes. However, the importance of global warming will also be a function of water availability, which depends in part on precipitation regimes that have unreliable predictions in most global circulation models developed for mountainous regions (Christensen *et al*. 2007). Threshold models were also used to predict that global warming, associated with an increase in winter and spring precipitation in the Canadian prairie, will likely increase germination of an endangered annual plant, *Cryptantha minima* Rydb. (Wei *et al.* 2009); while, a warmer and drier future climate may reduce regeneration success of the grass *Festuca hallii* (Vasey) Piper (Qiu *et al.* 2010). The information obtained may be also relevant for the study of long-term changes in *Polylepis besseri* demography in response to climate change. In this case it will be important to disentangle which life stages are the demographic bottlenecks for the establishment of *P. besseri*. Hydrothermal time models are an effective tool for studying germination ecology. The estimated parameters allowed us to discuss possible factors limiting germination in the field, to suggest the time required to perform germination studies for the species, and to make general predictions regarding the potential effects of global warming. HTT models can also give important information about population functioning if populations of different size or located in environmental, latitudinal or altitudinal gradients, were studied. In this sense HTT models re-emerge as very useful tools for studying germination ecology.

CHAPTER 4

Local adaptation of germination characteristics in *Polylepis besseri* and inferences regarding the consequences of global warming

Introduction

Local adaptation is a process resulting in a pattern called also local adaptation where resident genotypes have, on average, a higher relative fitness under their local environmental conditions, compared to genotypes originating from sites with different conditions (Williams 1966; Kawecki & Ebert 2004). Local adaptation is a complex phenomenon and has been shown to be influenced by gene flow, genetic drift, the species genetic variability, time since population establishment, and temporal and spatial environmental variability (Kawecki & Ebert 2004). Plants have been demonstrated to adapt to a range of local environmental conditions such as heavy metals in soils, the use of fertilizers and herbicides, differences in soil moisture, pollinating vectors, habitat fragmentation, temperature and elevation (reviewed in Linhart & Grant 1996; Bone & Farres 2001; Jacquemyn *et al.* 2012).

The use of reciprocal transplant experiments (where individuals originating from different populations are grown together in their original habitats and in the source habitats of the other populations (Kawecki & Ebert 2004)) has become a common approach to estimate the response of plant populations moving into new habitats (Antonovics 1976; De Frenne *et al.*

2011). Specifically in the study of local adaptation to elevation, the use of reciprocal transplant experiments across an altitudinal gradient allows the estimation of local adaptation and, indirectly, inferences regarding effects of global warming on plant populations (Byars *et al.* 2007; Giménez-Benavides *et al.* 2007; Gonzalo-Turpin & Hazard 2009; Ishizuka & Goto 2012).

Species from mountain ecosystems are expected to be very susceptible to global warming (Buytaert *et al.* 2011) because of the unprecedented rate of warming in the mountain regions (Bush *et al.* 2004), the rise in the elevation of snow cover, altered river discharge regimes (Schröter *et al.* 2005), and the narrow habitat tolerances of the mountain flora in conjunction with marginal habitats for many species (Thuiller *et al.* 2005). In mountains, the reported consequences of climate change include disrupted interactions among species (Didion *et al.* 2011; Brodie *et al.* 2012) and changed community composition (Peñuelas & Boada 2003) as a result of changes in the phenology (Bradley *et al.* 1999; Menzel *et al.* 2006; Parmesan 2006) and elevation shifts (Parmesan & Yohe 2003; Lenoir *et al.* 2008). Although it has been suggested that mountains have enough heterogeneity in topography to buffer global warming effects (Scherrer & Körner 2011), it is clear that if not pre-adapted, mountain plant species need to move, either horizontally or upwards, to reach new and suitable sites for their re-establishment (Parmesan 2006; Byars *et al.* 2007; Atkins & Travis 2010; Chmura *et al.* 2011). A final option for plant species is to adapt to the new environmental conditions in their current locations, and although local evolutionary responses to climate change have occurred with high frequency, there is no evidence so far for a change in the absolute climate tolerance of a species under natural conditions (Parmesan 2006; Gienapp *et al.* 2008; Hoffmann & Sgrò 2011; Dawson *et al.* 2011).

In the face of global warming, seed germination is a key process because it is a critical bottleneck for plant establishment (Walck *et al.* 2011). It initially acts as a sieve for range shifts, and when successful, it determines the future conditions that plants will experience. It is subjected to natural selection, and influences the phenotypic expression of postgermination traits (Donohue *et al.* 2010). Additionally, it was found that an early-germinating strategy produced a higher percentage of seedling survival and a higher number of seeds produced per plant compared to a late-germinating strategy (Baskin & Baskin 1998). In fact, based in 51 studies, Verdú & Traveset (2005) found that early emergence can enhance plant fitness via increasing survival, growth and/or fecundity. Moreover, this increase in fitness can last for many years after germination in perennial plants (*e.g.* De Luis *et al.* 2008). Germination was also studied among other fitness components to determine the adaptation of *Clarkia xantiana* A.Gray (Onagraceae) across a subspecies border (Geber & Eckhart 2005). Thus, we can state that germination can be used as a fitness component, and that it is particularly important in long lived species (such as trees) because of the long time required to monitor their survival, growth, and fecundity. Next to evaluating local adaptation of germination in the field through reciprocal transplant experiments, laboratory experiments can help to increase insight into this process. Hydrothermal time models can predict how any specific fraction of a seed population will respond to alterations in environmental conditions (Bradford 2002), and thus they can be applied to study the response of plant species to climate change (see Chapter 3).

The initial effects of climate change may be somewhat buffered by biotic interactions. Species interactions involve a complex balance of competition and facilitation that may vary with the life stages of benefactors and beneficiaries (Callaway & Walker 1997). Competition is the consequence of sharing limited resources, and interference is the result of the release of chemicals to the environment that will harm nearby plants (*i.e.* allelopathy, Padilla & Pugnaire 2006). Both processes result in negative plant-plant interactions and are dominant in mild abiotic conditions (Callaway *et al.* 2002). In contrast, positive plant interactions (*i.e.* facilitation, Padilla & Pugnaire 2006) are strong where abiotic stress is high (Callaway *et al.* 2002) and when beneficiaries are young and small (Callaway & Walker 1997). In some habitats, seedling establishment may be enhanced near adult plants that ameliorate extreme environmental factors (Padilla & Pugnaire 2006). High elevation ecosystems are characterized by severe and frequently life-threatening abiotic conditions that change dramatically even on a daily basis

(Alonso-Amelot 2008). Thus, facilitation may be an important plant-plant interaction for the establishment of new individuals. Moreover, Andean mountain temperatures decrease by 0.55°C when altitude increases 100m (Bush *et al.* 2004), thus decreasing elevation *c.* 200m would result in 1.1°C increase in temperature. This increase in temperature is very likely to occur between the present and 2080-2099 due to global warming (Christensen *et al.* 2007), and may disrupt the balance between positive and negative plant-plant interactions.

Little is known about the effects of global warming on the germination of *Polylepis* species (Cierjacks *et al.* 2007b; Marcora *et al.* 2008). The aim of the present study was to combine reciprocal transplant experiments and hydrothermal time modelling to detect possible local adaptation of *Polylepis besseri* seed germination characteristics to altitude. More specific, we asked the following questions: 1) how is seed germination success changing when seeds are introduced at different altitudes? 2) Given the extreme abiotic conditions in the high Andes, is facilitation the dominant plant-plant interaction at different elevations? 3) Do seeds from different altitudes have different germination requirements as quantified by their hydrothermal time model parameters?

Materials and methods

Study species and study area

The study species (*Polylepis besseri* Hieron.) and the study area (Sacha Loma and Cuturi), were already described (see Chapter 3). The study area has a pluviseasonal climate (Fernández *et al.* 2001; Fig. 4.2) with the growing season from November to March, and the dry and cold season from June to August (Gareca *et al.* 2010a, see Chapter 5). Based on the climatic data from the nearest meteorological station (Arani, which is 30km away and 1000m below the study site), we estimated a mean annual temperature of 12°C (corrected with a 5.5°C decrease per 1000m increase in elevation; Bush *et al.* 2004), and a mean annual precipitation of 325mm for the period 1990-2009

(Fig. 4.2, *segmented lines*). A higher (*i.e.* +39mm) precipitation than usual in November and January, and a lower precipitation in March and April (*i.e.* -20mm and -13mm, respectively) characterized the growing season of the year 2009-2010 (Fig. 4.2, *continuous lines*).

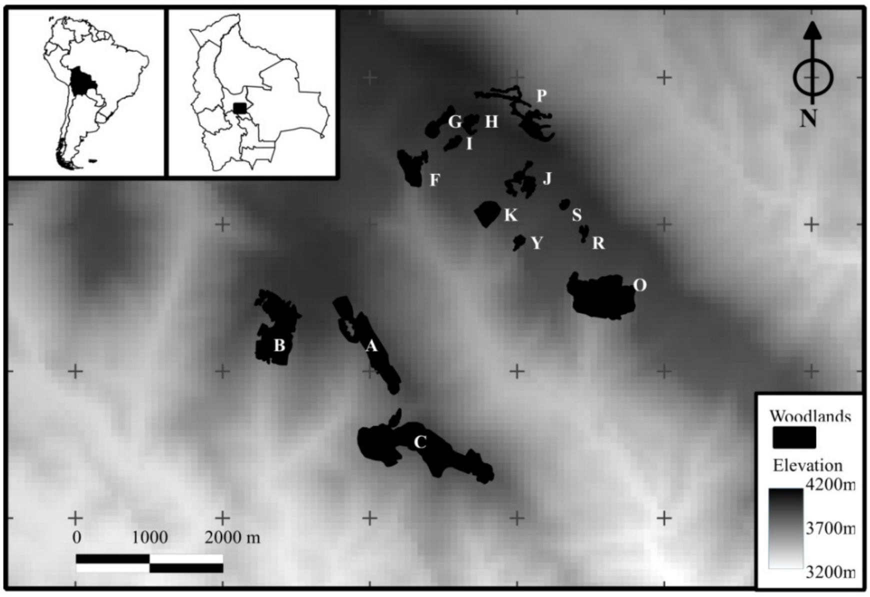

Fig. 4.1 Location of the study area (*insets*). *Polylepis besseri* woodlands are in *black*. *Letters* represent woodland codes. Shading refers to topography, where *white* represents elevations <3200m and *dark gray* elevations of 4200m.

Fruit collection

Fruit collection took place in December and January 2009-2010 and 2010-2011. In order to avoid possible inbreeding effects, two large woodlands were selected for fruit collection, woodland B and O (Fig. 4.1). Mature fruits were collected from *c*. 150 randomly selected trees from both the highest and lowest elevations of *P. besseri* occurrence in both fragments (3600 and 3800m a.s.l.). These elevations represent the source of potential mother trees in the area and the evaluation of their germination characteristics is important for future reforestation programs. Fruits were cleaned with a brush, selected to avoid predated fruits, and mixed by altitudinal origin (*i.e.* combining the fruits from both fragments). Because of the large number of fruits required to perform the experiment (*i.e.* 18,000, see below), it was not possible to keep the fragment of origin as a factor (*i.e.* not mixing fruits from both fragments). Since fruits are the dispersal units, germination experiments were done using them (Baskin & Baskin 1998), but the term ‘seed’ will be used throughout this paper.

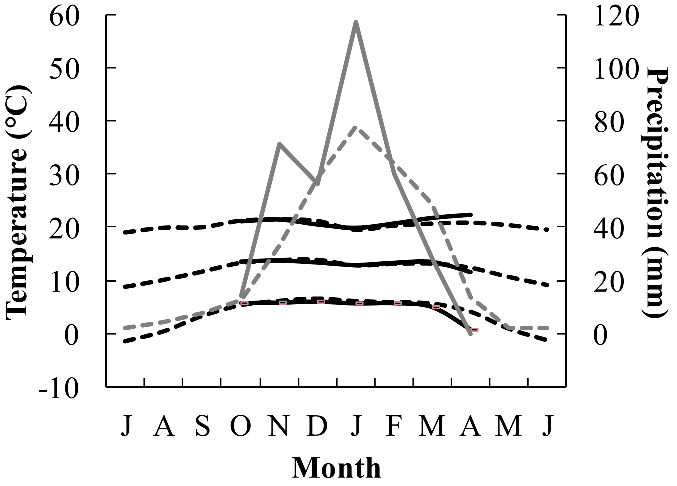

Fig. 4.2 Climadiagram for Sacha Loma and Cuturi (3750m a.s.l., Bolivia) since 1990 to 2009 (*dashed lines*) and since October 2009 to April 2010 (*continuous lines*), showing the maximum, mean and minimum temperatures (*black lines*) and monthly precipitation (*gray lines*).

Reciprocal transplant experiment

The field experiment took place during the growing season 2009-2010. Three transects were established, covering the altitudes 3800, 3600 and 3400m. The altitude of 3400m falls outside the *Polylepis* range in the study area and was selected as to represent a warmer condition than 3600 and 3800m, where *P. besseri* is naturally occurring. These 400m difference between 3800m and 3400m represents an increase of 2.2°C (Bush *et al.* 2004), likely to occur in the high Andes between the present and the period 2080-2090 (Christensen *et al.* 2007), and that may disrupt the balance in plant-plant interactions. Two transects were located in Sacha Loma (woodlands A and C, Fig. 4.1) and one in Cuturi (woodland O, Fig. 4.1). We did not have the owner's permit to perform the experiments in woodland B, thus we could not establish any transects there. All transects had a predominant South exposition. In each transect, 12 treatments were established. Each treatment was the factorial combination of three sowing elevations (3400, 3600 and 3800m), two altitudinal origins of the seeds (3600 and 3800m) and a facilitation effect (measured as with nurse and without nurse plant). The with-nurse treatment was established sowing *P. besseri* seeds next to the trunk of a *P. besseri* tree (at 3600 and 3800m), and next to the shrub *Brachyotum* sp. (Melastomataceae) (at 3400m where no *Polylepis* was present). We assume both species will have similar facilitation effects (*i.e.* protect against harsh conditions, Padilla & Pugnaire 2006). The no-nurse treatment was applied in nearby grassland, at least 15m away from the crown perimeter of the nurse plant. We did not apply the no-nurse treatment in gaps within *Polylepis* woodlands because we wanted to study the potential for colonization of the surrounding grassland. This grassland is composed of *Festuca, Stipa* and *Calamagrostis* species which can reach 60cm in height (Fernández *et al.* 2001, see Chapter 6) and their cover is highly variable. The experimental units were perforated plastic bags containing sieved local soil and 500 seeds from the corresponding altitudinal origin that were covered with a thin layer of soil. A total of 18,000 seeds were sown at the end of January 2010. Each of the 36 experimental units was covered with a stainless metal mesh to avoid predation, and evaluated every three weeks (during 12wk), counting and removing germinated seedlings. Only 27 experimental units could be retrieved at the end of the experiment, the other nine were destroyed by unknown causes.

Hydrothermal time modelling

In order to assess how seed germination characteristics changed with increasing the elevation origin of seeds, a laboratory experiment was performed with fruits collected from 3600 and 3800m a.s.l. in December 2010 and January 2011. Fruits were cleaned with a brush, and in October 2011, seeds from each elevation were placed in Petri dishes. A total of 12 treatments were applied to each set of seeds. The treatments resulted from the factorial combination of three water potentials (0.0, -0.3 and -0.6MPa) and four temperatures (5, 10, 15 and 20°C). Each treatment had three replicates; each replicate consisted of five Petri dishes with 25 fruits each, making a total of 125 fruits per replicate. Treatments were selected to allow the estimation of the hydrothermal time model parameters below the optimum temperature of *P. besseri* (*c*. 22°C, see Gareca *et al*. under review, Chapter 3). The preparation of the water potentials followed Gareca *et al*. (under review, see Chapter 3) based on Michel (1983). Each Petri dish was covered with parafilm to avoid evaporation. The germination was evaluated every week during 12wk; germinated seeds were removed after each evaluation. Water potential solutions were renewed after the 5th and 9th week.

Data analysis

The selected response variables for the reciprocal transplant experiment were 'total germination after 12wk' and 'germination rate', which was estimated as the inverse of the time to reach 50% of the total germination ($1/t_{50}$). Both variables were analyzed with general mixed models using the MIXED procedure (SAS Institute Inc. 2008). Since the three studied transects were selected randomly from a population of possible transects, they were treated as a random factor. Total germination was used as such, whereas $1/t_{50}$ was square root transformed to satisfy the normality assumption. The models for both response variables included the main effects transect (t, random), origin of fruits (o, fixed), sowing elevation (s, fixed) and nurse effect (n, fixed), as well as the double and triple interaction for the fixed effects. Since some factors had F-values < 1, they were removed from the analysis, leading to model reformulation as follows: total germination was modeled as: t + s + n + s x n + s x o + n x o + s x n x o, whereas $1/t_{50}$ was modeled as t + s + o + s x o. If significant differences were found, contrasts with one degree of freedom were analyzed between the treatments. The interaction s x o was analyzed in two ways: First, to test for local adaptation of seed germination at both elevations the 'local *vs.* foreign' criteria was used (Kawecki & Ebert 2004), where the germination of seeds from 3600m (foreign) and 3800m (local) were compared when sown at 3800m, and the germination of seeds from 3600 (local) and 3800m (foreign) were compared when sown at 3600m. Second, to test for the global warming effect, the ‘home *vs.* away’ criteria was used (Kawecki & Ebert 2004). Therefore, germination of seeds originating from 3600m was compared when germinated at 3600m (home) *vs*. 3400m (away). Whereas the germination of seeds from 3800m was compared when germinated at 3800m (home) *vs*. 3600m (away) and 3400m (away).

The results from the laboratory experiment were summarized by the cumulative percentage of germination per week. Then, the hydrothermal time model parameters below the optimum temperature for seeds from both origins (3600 and 3800m a.s.l.) were obtained using the methodology described in Gareca *et al.* (under review, see Chapter 3) based on Gummerson (1986), Bradford (1990), and Alvarado & Bradford (2002). To test for the differences in germination between seeds from both elevations, both a numerical and a graphical comparison between the patterns of germination were done.

Results

Reciprocal transplant experiment

Sowing elevation, the interaction sowing elevation x origin of fruits, and nurse effect significantly affected the total germination after 12 weeks (Table 4.1). Because the effect of sowing elevation depended on the origin of fruits (significant interaction sowing elevation x origin of fruits), the main effects of sowing elevation were not evaluated. The interaction term (sowing elevation x origin of fruits) showed two patterns. First, local *P. besseri* seeds at 3800m germinated *c.* 50% more than the foreign seeds (*i.e.* seed origin 3600m) at that elevation (Fig. 4.3, $P = 0.02$); this pattern was not found for seeds sown at 3600m, where local and foreign seeds performed similarly ($P = 0.29$). Second, seeds from 3600m germinated 36% more at 3400m than at their home site ($P = 0.05$); while seeds from 3800m germinated 65% more at their home site, compared to 3600m ($P = 0.01$), but the total germination was similar for the home (*i.e.* 3800m) and the away site at 3400m (*P* = 0.32). Regarding the germination rate (1/t_{50}), the only significant effect was the sowing elevation (Table 4.2). Germination rate decreased with increasing sowing elevation (Fig. 4.4). The nurse plants had a negative effect on the total germination; seeds without nurse plants exhibited 30% more germination than seeds with nurse plants (Fig. 4.5).

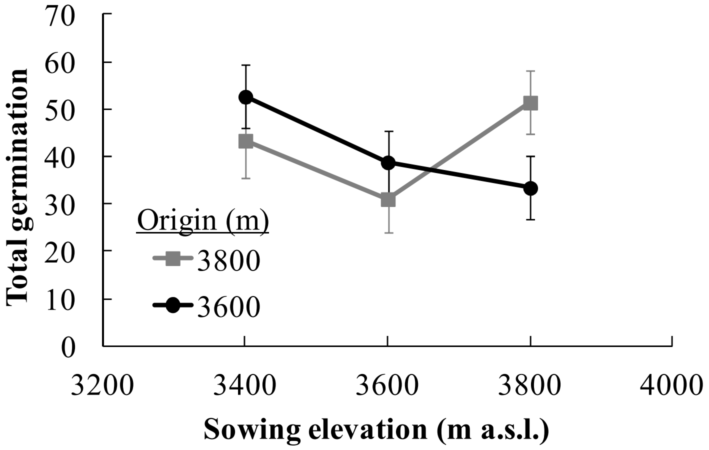

Fig. 4.3 Mean effects of sowing elevation and seed origin in the total germination of *Polylepis besseri* after 12wk of field experiment in Sacha Loma and Cuturi (Bolivia). *Bars* represent standard errors.

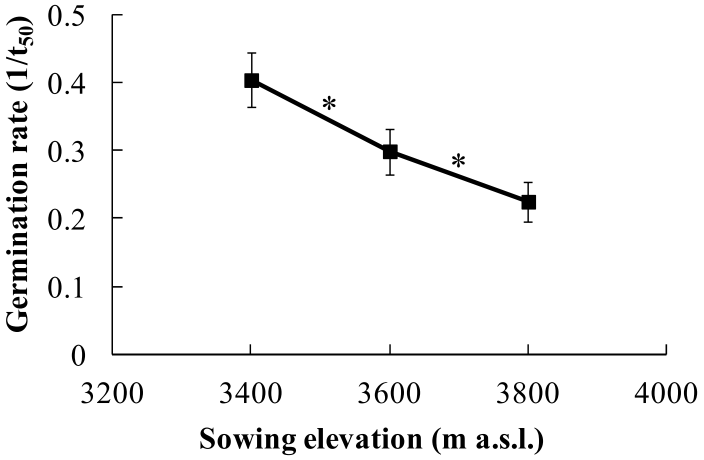

Fig. 4.4 Germination rate (1/t_{50}) as function of sowing elevation for *Polylepis besseri* seeds after 12wk of field experiment in Sacha Loma and Cuturi (Bolivia). * indicates significant differences ($P < 0.05$). *Bars* represent standard errors.

Table 4.1 Analysis of variance for the total germination of *Polylepis besseri* after 12wk of field experiment in Sacha Loma and Cuturi (Bolivia).

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F value	*P* > F
Sowing elevation [s]	2	13.1	4.13	0.04
Nurse effect [n]	1	13.1	8.65	0.01
s x n	2	13.4	1.71	0.22
s x origin of seeds [o]	2	13.3	6.33	0.01
n x o	1	13.1	2.93	0.11
s x n x o	2	13.2	2.84	0.09

Table 4.2 Analysis of variance for the germination rate ($1/t_{50}$) for *P. besseri* after 12wk of field experiment in Sacha Loma and Cuturi (Bolivia).

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F value	*P* > F
Sowing elevation [s]	2	19.1	21.0	0.01
Origin of seeds [o]	1	19.3	3.9	0.06
s x o	2	19.3	1.0	0.34

Table 4.3 Parameters of the hydrothermal time models for *Polylepis besseri* for an optimum temperature of *c*. 22°C (Gareca *et al*. under review, see Chapter 3) and for both seed origins studied (3600m and 3800m a.s.l.). The parameters obtained previously for the same species were also reported (Gareca *et al*. under review, see Chapter 3).

Seed origin (m a.s.l.) or study	T (°C)	ψ (MPa)	θ_{HT} (MPa·°·w)	T_b (°C)	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	R^2
3600	5-20	-0.6-0	24.5	2.7	-0.85	0.34	0.92
3800	5-20	-0.6-0	25.1	2.6	-0.86	0.34	0.87
Gareca *et al*. (under review, see Chapter 3)	5-20	-0.9-0	23.0	3.0	-0.74	0.41	0.78

Hydrothermal time modelling

Based on the germination cumulated at different water potentials and temperatures for seeds from both origins, the hydrothermal time model parameters determined for *Polylepis besseri* seeds from the two studied elevations were very similar (Table 4.3). The hydrothermal time required for germination (θ_{HT}), *i.e.* the time to reach germination for a given seed fraction or percentage *g* when expressed on a hydrothermal time basis was *c.* 25MPa·°C·w. This means that to germinate a seed needs to accumulate a certain amount of temperature above the base temperature wich was *c.* 3°C, and a certain amount of water potential above the base water potential for a specific seed fraction *g*. Because the base water potential is specific for each seed fraction *g, i.e.* it changes according to a normal distribution with a mean or base water potential for 50% of the germination ($\psi_{b(50)}$) of -0.85MPa and a standard deviation of 0.34MPa ($\sigma_{\psi b}$), the base water potential needs to be calculated for each seed fraction *g*. The product of these amounts of thermal and hydrotime cumulated above the corresponding thresholds with the time required to reach a certain percentage of germination is a constant known as the hydrothermal time constant θ_{HT}. For example, using the parameters estimated for seeds from 3600m (Table 4.3) and if the temperature and water potential that seeds experience were 20°C (T) and -0.3MPa (ψ), respectively; and if we wanted to know the time it will take to germinate 70% of these seeds (*g*), we can estimate the amount of temperature above the base temperature (*i.e.* 20-2.7= 17.3°C) and the amount of water potential above the base water potential for *g* = 70%. For the latter, we need to estimate the base water potential for *g* = 0.7, this is the inverse of the cumulative normal distribution with mean -0.85MPa and standard deviation 0.34MPa, and equals -0.67MPa. The amount of water potential above the base water potential for *g* = 0.7 is 0.37MPa (*i.e.* -0.3-(-0.67) = 0.37MPa). Now we can estimate the time to reach 70% of germination as 25MPa·°C·w / (0.37MPa*17.3°C), which yields 3.8w. Furthermore, if we want to normalize the germination time course of any water potential into the germination time course for seed germination in water, we need to multiply the time to germination (*i.e.* 3.8w) by the correcting factor $1-(\psi/\psi_{b(g)})$ (eq. 7, Bradford 2002), this equals 2.1w and to put it on a normalized thermal time scale we need to multiply it by the amount of temperature above the base temperature (*i.e.* 17.3°C), which yields 36.5°C·w. This is the corresponding value to reach 70% of germination on a normalized thermal time in pure water and can be seen in the predicted germination percentages in Fig. 4.6a.

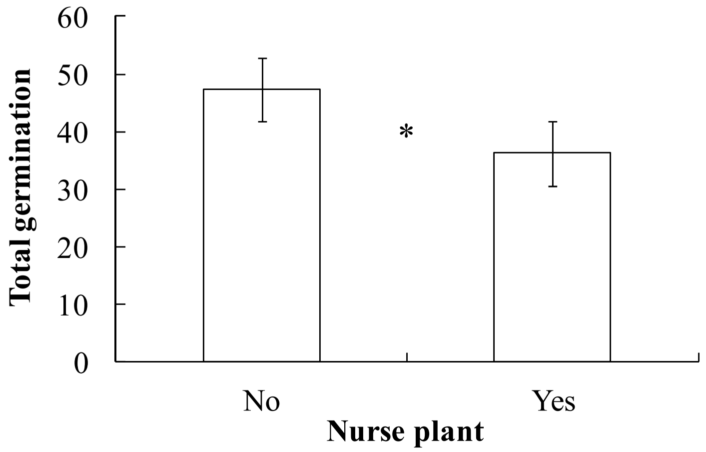

Fig. 4.5 Nurse plant effect (*Polylepis besseri* or *Brachyotum* sp.) on the total germination of *Polylepis besseri* after 12wk of field experiment in Sacha Loma and Cuturi (Bolivia). * indicates significant differences between treatments ($P < 0.05$).

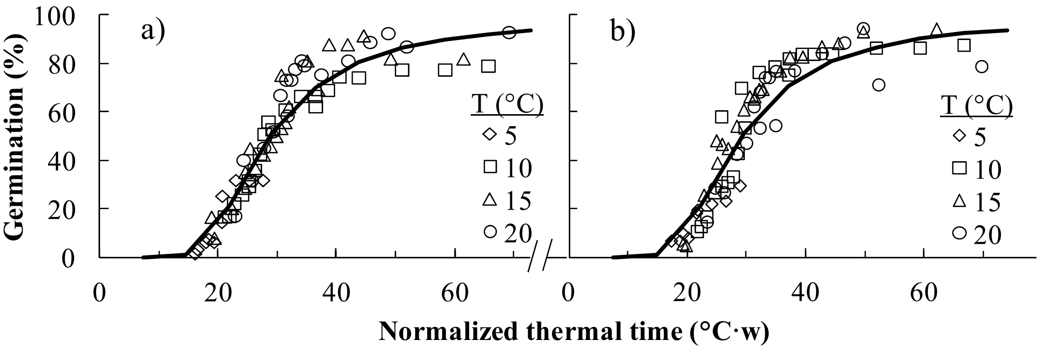

Fig. 4.6 Observed (*symbols*) and predicted (*lines*) germination percentages as function of the normalized thermal time for seed origin a) 3600m and b) 3800m a.s.l. in pure water (ψ = 0.0MPa).

Discussion

Despite the short differences in elevation in this study, we found differences in germination rate and total germination of *Polylepis besseri*. We found evidence for possible local adaptation in *Polylepis besseri* seed germination to high elevations. This pattern was observed even in the presence of gene flow in the study area (see Chapter 7). The pattern was not found when seeds were germinated at lower elevations. Germination rate decreased with increasing sowing elevation, and germination was higher in the grassland than under a nurse tree or shrub. We also found similar hydrothermal time model parameters for seeds from 3600 and 3800m. These results show that: 1) the trend of changes in *P. besseri* seed germination depends on two factors: the source elevation of the seeds and the sowing elevation; 2) the presence of a nurse tree or shrub did not facilitate the germination of *P. besseri*, or grasses equally functioned as nurse plants; and 3) seeds from both elevations may germinate similarly under most lab conditions.

Seeds from 3600m diminished their total germination as sowing elevation increased (Fig. 4.3, *circles*). Thus, they had a better germination in milder temperature conditions (3400m) than under harsh conditions (3800m). This pattern has a straightforward explanation since at low elevations (higher temperatures) seeds may accumulate more thermal time required for germination (see Chapter 3). As in our study, the number of *P. incana* seedlings decreased with increasing elevation, likely due to more extreme climatic conditions at high elevations (Cierjacks *et al.* 2007b). However, *P. australis* seeds had the opposite pattern of germination, they increased their percentage of germination as elevation increased (*i.e.* decreasing temperature) (Marcora *et al.* 2008). *P. australis* pattern was suggested to be the result of longer filling periods of seeds and higher soil moisture at high elevations, whereas longer periods of drought at lower elevations might affected embryo development and seed mass (Marcora *et al.* 2008). While *P. australis*' regeneration pattern suggests that an increase in temperature due to global warming may restrict it to its uppermost distributional altitudes (Marcora *et al.* 2008), the study on *P. incana*, *P. pauta* (Cierjacks *et al.* 2007b) and this study on *P. besseri* suggest that an increase in temperature may increase *Polylepis* regeneration, at least if seeds from 3600m are used.

On the other hand, seeds from 3800m showed their maximum total germination at low (3400m) and high elevations (3800m, *squares* in Fig. 4.3). The germination of *P. besseri* seeds from both elevations at 3400m (Fig. 4.3) shows the capacity of the species to cope with warm conditions such as those present at that altitude. If we had studied the seed germination at 3400 and

3600m, we could say that total germination decreased with increasing elevation in seeds from both seed origins. However, when including the 3800m sowing sites in the picture, we can see that seeds from 3600m continue with the pattern of decreasing total germination with elevation, whereas the seeds from 3800m present an abrupt change in the pattern: they increased their germination at their home site (3800m). This increase in germination explains the strange dip at 3600m for seeds from 3800m. The home site advantage observed for these seeds suggest a pattern of local adaptation of *P. besseri* to high elevation. Although the study was limited to one growing season, this pattern suggests that selection may have resulted in genotypes adapted to high elevations, and possibly colder conditions, given all the studied transects had a predominant South exposition. The pattern of local adaptation of reproductive traits or survival to high elevations has been found for *Poa hiemata* (Byars *et al.* 2007), *Festuca eskia* (Gonzalo-Turpin & Hazard 2009), *Silene ciliata* (Giménez-Benavides *et al.* 2007), and *Abies sachalinensis* (Ishizuka & Goto 2012). Our results support the proposed hypothesis of *Polylepis* colonization in the Andes from warm and low elevation sites, to high and cold sites (Fjeldså & Kessler 1996; Schmidt-Lebuhn *et al.* 2010), and suggest local adaptation might be a process leading this pattern. In the face of global warming, the local adaptation to high elevation conditions might become a maladaptation to the warmer environments that *P. besseri* would encounter in the future, as was also reported for *Pseudotsuga menziesii* in the Pacific Northwest of the U.S.A. (St Clair & Howe 2007). An increase in temperature due to global warming may initially reduce the total germination of *P. besseri* seeds from 3800m.

These results do not mean we demonstrated local adaptation for total germination at high elevations because: (i) germination was the only life stage studied, and we do not know the contribution of germination to lifetime fitness of *Polylepis besseri*. It will be important to find out the contributions of the different fitness components (*e.g.* growth, survival, fruit set, seed set) to differences in total fitness among *P. besseri* individuals. (ii) We did not estimate the fitness for individuals that germinated at different times in the field experiment; and (iii) the observed pattern might be the result of maternal effects (Donohue *et al.* 2010). The mother plant may affect its seeds by one or more mechanisms: genetics, non-Mendelian inheritance (*e.g.* via chemicals produced by the mother), and/or through interactions with the environment (preconditioning) (Baskin & Baskin 1998). Competition, physiological age of plants, position on mother plant, soil moisture and temperature, all of them could cause changes in germination responses in the subsequent generations, thus preconditioning seed germination (Baskin & Baskin 1998). To avoid this, it is recommended to maintain samples from all populations under common environmental conditions for two or three generations before their fitness is measured (Kawecki & Ebert 2004). However, this is not possible for long lived specieas such as trees.

The decrease in germination rate with increasing elevation (Fig. 4.4) suggest that global warming will increase seed germination rate, therefore seedlings may appear early and grow more during the growing season (Verdú & Traveset 2005; De Luis *et al.* 2008). Moreover, seedlings that would tolerate the increase in 1.7 to 5.6°C predicted for the next century in the Andes (Christensen *et al.* 2007), might have an advantage that may last for years over other seedlings (Verdú & Traveset 2005). The effect of facilitation on germination rate could not be evaluated.

Seeds from one woodland were sown in their own woodland (*i.e.* woodland O), this might have given an advantage to these seeds because the seeds were already adapted to their abiotic conditions, and thus increased the germination in woodland O. However, we could not separate the fragment of origin factor from the seeds in the way the experiment was performed (*i.e.* mixing the seeds from both collected woodlands prior sowing them). The differences in germination caused by sowing the seeds in different transects (*i.e.* woodlands) and that may had reflect the home site advantage of seeds from woodland O, were corrected in the analysis by using transect as a random effect.

A higher total germination was observed in the grassland compared to the germination under a nurse plant in the absence of herbivores, and with enough seeds and microsites (Fig. 4.4). Although it has been found that plant facilitation increased with intensified stress in high *vs*. low altitudes in alpine environments (Callaway *et al.* 2002) and in Mediterranean mountains (Gómez-Aparicio *et al.* 2004), our results suggest a negative plant-plant interaction between *P. besseri* seeds and the potential nurse plants (tree and shrub). This negative interaction could be the result of intra and interspecific competition with *P. besseri* trees, and with the shrub *Brachiotum* sp.. The potential mechanisms shaping this pattern can be interference (allelopathy) or competition for resources such as space, water, nutrients and light (Padilla & Pugnaire 2006). Negative interactions have been found to be stronger than positive interactions under mild abiotic conditions (Callaway *et al.* 2002). It has been found that *P. pauta* and *P. incana* germination increased under the canopy of *Polylepis* trees and in sites with removed litter (Cierjacks *et al.* 2007a). These authors hypothesized that extreme microclimatic conditions in the outside grassland caused a lower germination in those areas compared to the inside and the edge of the woodlands (Cierjacks *et al.* 2007a). Whereas this study was performed in the upper limit of the altitudinal distribution of *P. incana* and *P. pauta*, ours was performed in the middle range of *P. besseri* altitudinal distribution, thus our climatic conditions might not be not as extreme for *Polylepis* as those studied by Cierjacks *et al.* (2007a). The possibility that grasses acted as nurse plants in the grassland cannot be discarded (*e.g.* Gasque & García-Fayos 2004).

Our results also suggest that *P. besseri* could colonize the grassland outside the *Polylepis* woodlands in the absence of herbivory, with available microsites and enough seeds for germination. It has been found that livestock with moderate grazing may promote *P. australis* recruitment likely because the species is adapted to a herbivore controlled ecosystem, since large herbivores were present before the introduction of domestic livestock (Zimmermann *et al.* 2009). Available microsites are also important for germination and may interact with the presence of livestock. In *P. incana* and *P. pauta* woodlands, it has been found that livestock trampling had a positive impact on seedling abundance, presumably due to the removal of the litter layer and the creation of safe sites for germination (Cierjacks *et al.* 2008a). Finally, if *P. besseri* seeds do not get into the grassland, they will not be able to germinate. The dispersal distance of *Polylepis* seeds seem to be restricted to a few meters from the mother trees (Cierjacks *et al.* 2007b; Torres *et al.* 2008) and no secondary dispersal agents such as rodents are known for any of the *Polylepis* species (Zimmermann *et al.* 2009). Thus, dispersal limitation might be an important limiting factor for the establishment of new *Polylepis* individuals in the grassland, with the consequences in population migration. It has been found that if tropical tree species continue migrating at the current rate of migration and cannot tolerate and/or adapt to rising temperatures, they will fail to keep pace with future climate changes (Feeley *et al.* 2011).

The reciprocal transplant experiment showed a pattern of local adaptation to high elevations, suggesting that seeds from 3800m have different germination characteristics compared to seeds from 3600m (Fig. 4.3). However, seeds from both elevations showed similar HTT model parameters (Table 4.3), suggesting that the germination characteristics of both seeds are alike. This difference might be explained by the overestimation of germination of the HTT models under temperatures below the base temperature for germination (see Chapter 3), in this case *c.* 2.7°C. HTT models could not accurately predict germination under conditions including temperatures below the base temperature for germination. Thus, experiments under constant and alternating temperatures that do not include temperatures below the base temperature (*e.g.* 20/10°C), do not necessarily reflect what occurs in nature. A second possibility is that seeds produced in different years (2009-2010 for the reciprocal transplant experiment, and 2010-2011 for HTT modelling experiment) present different germination characteristics, and therefore they germinate differently in the field and lab conditions. Finally, it is possible that trees from both elevations differ in the number of fruits with seeds inside or in the soil conditions around the seed tree. The reciprocal transplant experiment did not control for the difference in number of fruits without seeds since all healthy seeds were sown and no evaluation of the non-germinated seeds was done; whereas, the HHT model experiment did control for this difference because the germination percentages were based on available seeds only. Thus, the observed differences between both approaches might be due to this factor. In fact, the number of fruits with seeds from 3800m (mean 23.5) in the lab experiment was higher than the number of fruits with seeds from 3600m (mean 20.4, $F_{1,70} = 8.26$, $P = 0.01$). To remove the effect of the presence of seeds inside the fruits we could evaluate the presence of empty fruits at the end of the experiment, to scan the fruits previous to the experiment and select the ones with seeds, or to evaluate the weight of the fruits before performing the experiment and to correct for it in the data analysis. It has been found that relatively heavier fruits of *P. australis* have higher germination percentages than the light ones, and that the seeds that did not germinate were almost always empty (Seltmann *et al.* 2007a); if the correlation between fruit mass and seed presence is positive, then fruit mass could be used as a surrogate for seed presence and thus a way to control for the differences due to seed presence in the fruits. Regarding the soil conditions around the seed tree, it is known that *P. australis* seeds from sites with high soil erosion have lower seed viability than seeds from more conserved sites, may be due to low nutrients and/or water stress in the degraded woodlands (Renison *et al.* 2004); however, we did not observe differences in bare rock exposed due to soil erosion at the different elevations (Edgar E. Gareca, pers. obs.).

Based on our results, we can say that total germination of *P. besseri* will not be negatively affected by global warming because seeds from both origins had high total germination values at the lowest elevation (Fig. 4.3), which represented a global warming situation. What is clear is that the germination rate will increase while increasing temperature (*i.e.* decreasing elevation in Fig. 4.4). To give guidelines for conservation, it will be needed first to resolve if the local adaptation pattern on total germination is present in different years. In the face of global warming, seeds from high elevations (3800m) might become maladapted to warm conditions, given their home-site advantage for cold environments. The ability of seeds from low elevations to germinate under warmer conditions suggests that *P. besseri* could tolerate global warming, and translocation of individuals from low to high elevations might be recommended. We don't know whether other life-stages of *P. besseri* could cope with warm conditions the species would face in the future. It has been suggested that studies integrating all stages of the *P. australis* life cycle are necessary to gain a clear picture of its overall performance at increasing altitudes (Marcora *et al.* 2008). We also suggest the importance of studying the balance between positive and negative plant-plant interactions along *P. besseri*'s altitudinal distribution, and to perform germination experiments to disentangle which are the limiting factors for germination in the grassland (Turnbull *et al.* 2000). Long-term demographic monitoring, next to the assessment of genetic variability are needed to integrate current knowledge of climate change impacts across the species' ranges (Giménez-Benavides *et al.* 2007).

CHAPTER 5

Effects of exotic tree species on the regeneration of *Polylepis subtusalbida*

Adapted from Gareca, E.E., Y.Y. Martinez, R.O. Bustamante, L.F. Aguirre & M.M. Siles. 2007. Regeneration patterns of *Polylepis subtusalbida* growing with the exotic trees *Pinus radiata* and *Eucalyptus globulus* at Parque Nacional Tunari, Bolivia. *Plant Ecology* 193: 253-263.
The original publication is available at www.springerlink.com

Introduction

Plantations of exotic species are utilized for their wood-yielding, ornamental, and textile properties (Pimentel *et al.* 2000), that can be better than those yielded by native plants. The biology of native plants is often poorly understood, insufficient for use in economic analysis, and the causal factor is the unavailability of genetically improved native seed stock (Richardson 1998). Furthermore, some exotic plantations have the potential to maintain native plant and animal species because they may be used by wildlife as temporary refugees (Acosta-Jamett *et al.* 2003; Acosta-Jamett & Simonetti 2004); in this way, exotic tree plantations have been found to act as a viable solution for the recuperation of native vulnerable ecosystems (Lima 1987). However, exotic plants may negatively affect native species as well (Everett 2000). In fact, the invasion of the exotic tree *Acacia saligna* to the African fynbos biome reduced around 70% of the native vegetation (Holmes & Cowling 1997). Also plantations near areas of natural vegetation may reduce structural diversity, increase biomass production, and disrupt the prevailing vegetation and soil dynamics (Richardson 1998). For instance, *Eucalyptus* spp. may unbalance the hydrologic and nutrient cycles; they affect the surrounding plant and microorganism growth because of allelopathic effects, and reduce soil fertility (Jayal 1985 in Lima 1987). Among all of these effects, exotic plant species can change the dominance of native species. For instance, *Rhamnus frangula* favored the recruitment of shade tolerant seedlings and reduced the survivorship and growth of four species with different preferences for light (Fagan & Peart 2004) and the allelochemicals of *Eucalyptus* leaves reduce germination and growth of other native plant species (Fjeldså & Kessler 2004). These traits are not related with the fact that these species are exotics, they explain why these species are successful, both in their native range and outside it (Booth *et al.* 2003; Pyŝek & Richardson 2007).

This Chapter deals about the effects of the exotic *Pinus radiata* L. and *Eucalyptus globulus* L'Hér. on the regeneration of a native tree *Polylepis subtusalbida* (Bitter) M. Kessler & Schmidt-Leb (Kessler & Schmidt-Lebuhn 2006). This issue is of great importance for the understanding of the regeneration patterns of this native tree and for conservation purposes. Restoration of the high mountain *Polylepis* woodlands of South America has been highly recommended by the World Conservation Monitoring Centre (1998b, see Chapter 2) because they belong to one of the most endangered woodland ecosystems in the world. Moreover, *Polylepis* woodlands provide important ecosystem services (Fjeldså & Kessler 2004) and contain a large number of endemic species (Balderrama *et al.* 2006; Moya-Soto *et al.* 2006). This study is one of the firsts to consider the effects of exotic trees on the regeneration of *Polylepis* (but see Larrazabal 2004), and as such is extremely valuable. The hypothesis to be tested was that recruitment of *Polylepis*, as well as plant growth and survivorship, would be reduced by the presence of exotic trees. The population density, seedling growth rate, and survival of *P. subtusalbida* were compared between pure native woodlands and mixed forests, which contained native plus exotic trees at the southern slope of the Parque Nacional Tunari, Cochabamba, Bolivia.

Methods

Study area

Data for this study was collected at three locations in the Parque Nacional Tunari (PNT, 17°20'S, 66°10'W): Pajcha, Pintumayu, and Thola P'ujru (Fig. 5.1). PNT was created in 1962 to protect native vegetation, prevent erosion, provide protection against the development of gullies around the city of Cochabamba, and as recreational space (Rico 1996; Anon. 2001). The PNT comprises the mesothermic valley region and includes part of the ridge of the Tunari range (SERNAP 2000). The climate is temperate, with an average temperature range from –3.3 to 23.9°C and an average annual precipitation of approx. 600mm. The southern slope of the PNT extends through two regions: the valley slopes (in the semiarid mountains) and the highland (SERNAP 2000).

At the valley slopes near the highland, the region in which this study was conducted, the most representative species of the natural woody vegetation are *P. subtusalbida* (kewiña or queñua) and *Buddleja coriacea* (kiswara, Remy), and the grasses *Festuca* spp. (L.) and *Calamagrostis* spp. (Adans.). Humans used this native vegetation before colonial times (Capriles & Flores-Bedregal 2002), however, now, the exotic species, *P. radiata* and *E. globulus* are part of the arboreal stratum. It seems all of them were planted without the prior clearing of native species, may be because the native *Polylepis* woodlands were strongly degraded, but no description of the previous state of the native vegetation was found. Plantations were initiated from the early 70's in order to protect the city of Cochabamba from floods (Paz 1992). Of the total area of the southern slope of PNT (87,314ha), exotic plantations cover around 1500ha (1.7%) and the open native woodlands cover around 3500ha (3.9%) (CLAS 2002). The valley slopes landscape (3300–4000m a.s.l.) is a mosaic composed of grasslands, Andean croplands, rocks, and different types of forest fragments: pure *P. subtusalbida* (fragment-type K), fragments mixed with *P. subtusalbida* and *P. radiata* (fragment-type KP), fragments with *P. subtusalbida* and *E. globulus* (fragment-type KE), fragments with *E. globulus* and *P. radiata* (fragment-type EP); fragments with pure *P. radiata* (fragment-type P) and fragments with pure *E. globulus* (fragment-type E). The study areas that contained the whole fragment-types were Pajcha (Fig. 5.1C, 17°19’S, 66°08’ W), Pintumayu (Fig. 5.1B, 17°19’S, 66°09’ W), and Thola P’ujru (Fig. 5.1A, 17°17’S, 66°13’W).

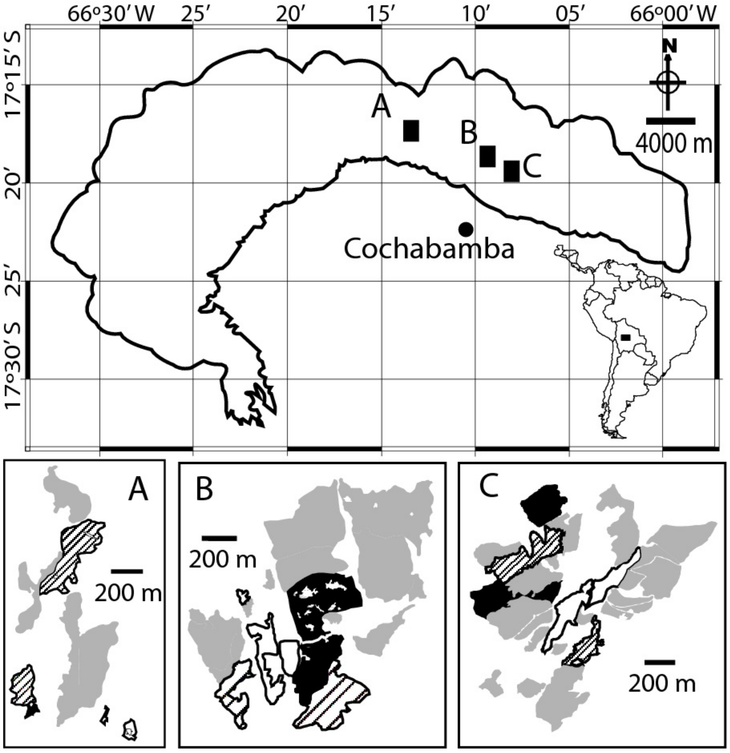

Fig. 5.1 Map of the study site. *Up*: Southern slope of the Parque Nacional Tunari and its location in South America. Locations are indicated by letters, (A): Thola P’ujru, (B): Pintumayu and (C):.Pajcha. *Bottom*: Fragment types in the three locations, *black areas* are *Polylepis subtusalbida–Eucalyptus globulus* fragments, *white areas* are mixed fragments of *P. subtusalbida–Pinus radiata*. *Hatched areas* are pure *P. subtusalbida* fragments, and *gray areas* are fragment types not used in the present study. Modified from Promic (2006) and Zárate (unpublished data).

People have used the three locations selected in different ways. Pajcha is mainly used for ecotourism, and to a minor degree for sheep-raising and agriculture; Pintumayu is used for agriculture by villagers living ~2000m downhill from the nearest woodland. The last location, Thola P’ujru, is used for agriculture and cattle-raising with a village located ~200m from the nearest woodland. Three fragment-types were selected at each location: fragment-type KP, fragment-type KE, and fragment-type K. Pure plantations were not studied because no *P. subtusalbida* seedlings are found there. A summary of the studied fragments is in Table 5.1. Current fragment sizes of the studied fragment-type K were between 3.0-7.8ha, of fragment-type KE 1.0-11.7ha, and of fragment-type KP 0.3-4.5ha (Table 5.1). The arboreal coverage in the mixed fragments was higher than in the pure fragment-type K due to the presence of *E. globulus* and *P. radiata* in the arboreal stratum. The adult density and basal area of *P. subtusalbida* in the pure fragment-type K was higher than in the mixed stands with exotic species, supporting the idea that the native vegetation was degraded before plantation; however, the number of death *P. subtusalbida* trees was higher in the stands with exotic species (Edgar E. Gareca, pers. obs.), so death by competition with *P. radiata* and *E. globulus* cannot be discarded. Both exotic species were 4 to 5 times higher than the native *P. subtusalbida* trees, thus competition by light can be the reason to find death *P. subtusalbida* trees. The basal areas of the exotic species were also higher than the basal areas of *P. subtusalbida* in the studied fragments (Table 5.1).

Sampling plots

At least two fragments out of every forest type were selected at each location. In each fragment, between three and five randomly located permanent plots of 10x10m were installed. In each permanent plot, all seedlings of *P. subtusalbida* ranging from 10 to 140cm in height were labeled. This size range was selected because we considered that individuals within this range are more susceptible to biotic/abiotic mortality factors than individuals of greater height, considering the 1yr temporal scope of this study. Altogether, 17 fragments and 64 plots were sampled across the three locations (Table 5.1). Abandoned croplands (frequently found within the forest fragments) and ravines were not selected for this study. In Thola P'ujru, because of the limited number of fragments (Table 5.1), only two permanent plots were established in each of the two mixed fragments (fragment-types KE and KP)

Table 5.1 Attributes of fragment-types (K: *Polylepis subtusalbida*, E: *Eucalyptus globulus* and P: *Pinus radiata*) for each location[a].

Location	Fragment type	Fragments studied	Fragment size[b] (ha)	Plots per frag.	Slope (%)	Arboreal coverage[c] (%)	Height[c] (m)		Basal Area[c] (m^2/ha)		Adult[c] density/ha	
							K	*E* or *P*	*K*	*E* or *P*	*K*	*E* or *P*
Pajcha	KE	2	11.7, 4.5	5, 4	26	52	3.3	12.7	3.8	21.8	217	717
17º19’ S	KP	2	4.5, 3.6	5, 4	24	80	3.3	17.1	4.1	35.3	242	417
66º08’ W	K	2	6.7, 2.2	4, 3	22	18	2.4	-	13.4	0	854	0
Pintumayu	KE	2	9.8, 5.5	3, 3	28	58	3.4	17.0	15.7	18.5	675	567
17º19’ S	KP	3	3.9, 1.9, 0.5	8, 5, 1	33	78	3.3	14.1	9.9	31.7	433	758
66º09’ W	K	2	7.8, 3.4	4, 3	37	36	4.2	-	26.1	0	850	0
Thola P’ujru	KE	1	1.0	2	40	54		>10				
17º17’ S	KP	1	0.3	2	11	76		>10				
66º13’ W	K	2	5.4, 3.0	4, 4	31	28					569	0
Total		17		64								

[a] Mean slope, arboreal coverage, adult height, basal area and density, are shown for each fragment-type. In all cases diameter > 9.9cm.

[b] Fragment sizes obtained from Promic (2006) and from us.

[c] Data for Pajcha and Pintumayu taken from Peredo (2004), and data for Thola P’ujru from Zárate *et al*. (unpublished data) and from us.

Data collection

The density of *P. subtusalbida* seedlings was evaluated from August 2004 to October 2005. During August 2004, all seedlings between 10 and 140cm in height in each permanent plot were labeled and their geographic coordinates were recorded. According to their growth form and size, evaluated as the height of the individuals, the seedlings were characterized in order to determine their population structure. For the growth form, the seedlings were classified in two categories: (1) vertical growth (V) if the dominant growth was apical, and (2) horizontal, if the dominant growth was from lateral branches. Within the latter category, the individuals whose lateral branches touched the ground and showed adventitious roots (LR) were differentiated from the individuals that did not show adventitious roots (L). The adventitious roots can offer information about the potential for asexual reproduction of *P. subtusalbida*. In order to analyze the size structure of seedlings, the individual height from the base of the trunk to the extreme of the branch that was leading the growth was measured; this branch could be vertical or lateral (Renison *et al.* 2002). The seedlings were grouped together according to their heights in: 10–41cm (class 1), 42–73cm (class 2), 74–105cm (class 3), and 106–140cm (class 4). Altogether, 1067 seedlings of *P. subtusalbida* were labeled.

The growth and survival of *P. subtusalbida* were measured, in a period of approximately 1yr. The growth in height was estimated as: $c = (h_1 - h_0)/h_0$ where c is the relative change in individual size, h_1 the seedling height in the 2005 sampling period and h_0, the seedling height in the 2004 sampling period. Survival rates were determined over the year of study.

Statistical analysis

Locations and fragment-types were considered as fixed factors for all of the analyses. The reason for treating location as a fixed factor was that the required habitats for proving the hypothesis were only found in Pajcha, Pintumayu, and Thola P'ujru (*i.e.* locations were not selected at random from a population of locations). The following nested analyses were performed due to the way we stratified the sampling procedure. For the analysis of growth rates, seedling density and the seedling survival, the fragments were nested in the interaction fragment-types by location, in order to use this source of variation as the term of error for the F-tests of location, fragment-type, and location x fragment-type effects (Quinn & Keough 2003). The analyses were accomplished using the procedures Genmod, Catmod, and Mixed on SAS software v. 8.00 (SAS Institute Inc. 1999).

Seedling density

The seedling density data were fitted to a negative binomial distribution because their variance did not increase as a function of their mean (Quinn & Keough 2003); therefore analyzed according to the generalized linear model theory (Littell *et al.* 1996) using the Genmod procedure and the Wald's statistic on SAS software v. 8.00 (SAS Institute Inc. 1999). Prior to the analysis of variance, the correlation between density of adult *Polylepis* woodland trees and seedling density was performed. If there was association between these variables then an analysis of variance with density of adult *Polylepis* woodland trees as a covariate was performed. To compare the mean density of *P. subtusalbida* seedlings between pure fragments (K) *vs.* mixed fragments (KP and KE), contrasts with one degree of freedom were developed.

Growth form and size structure

Since, the growth form structures of *P. subtusalbida*, which were classified in three categories or classes: vertical growth (V), lateral growth without adventitious roots (L), and lateral growth with adventitious roots (LR), and size structure, separated in four classes: 1, 2, 3 and 4 (see above), followed a multinomial distribution (any individual may belong to only one class), they were analyzed according to the generalized linear models theory (Littell *et al.* 1996). The odds of occurrence of every category were expressed as the class to which belongs any individual (*i*) in relation to the last class (*l*) in the form of generalized logits π_i / π_l using the Catmod procedure and the Wald's statistic on SAS software v. 8.00 (SAS Institute Inc. 1999). The last category (*l*) of the lifeform structure corresponded to plants with lateral growth and adventitious roots (LR), and the last category for the size structure was the largest size class (class 4). Afterwards, for the growth form structure, comparisons between the forest types K *vs.* KE and K *vs.* KP were performed at each location, developing contrasts with two degrees of freedom. While for the size structure, Pajcha *vs.* Pintumayu and Pintumayu *vs.* Thola P'ujru were compared with contrasts with three degrees of freedom.

Growth rate

The growth rate (*c*) comparisons between locations and fragment types were carried out through the following steps: (1) Ranges were assigned to the c variable because the data did not satisfy the normality and homogeneity of variance assumptions required according to the general linear model theory. (2) An analysis of variance was carried out to the ranges using the theory of the mixed linear models with the Mixed procedure and Satterthwaite's method for estimating degrees of freedom on SAS software v. 8.00 (SAS Institute Inc. 1999). (3) Contrasts with one degree of freedom were performed for the following paired fragment-type comparisons within each location: K *vs.* KE and K *vs.* KP. Furthermore, a comparison of the growth rates between size classes was performed. This comparison was similar to the comparison between locations and fragment-types stated before and the comparisons made were: classes 1 *vs*. 2, classes 2 *vs*. 3 and classes 3 *vs*. 4.

Seedling survival

Seedling survival, evaluated as alive or dead, followed a binomial distribution and was analyzed according to the generalized linear models theory using the Genmod procedure and Wald's statistic on SAS software v. 8.00 (SAS Institute Inc. 1999). If significant differences were detected, the following paired comparisons were carried out by developing contrasts with one degree of freedom: V *vs.* L and V *vs.* LR for comparisons between growth forms, and K *vs.* KE and K *vs.* KP for comparisons between fragment types. Furthermore, if significant differences were detected, a comparison of the survival between size classes was accomplished and the comparisons made were: classes 1 *vs*. 2, classes 2 *vs*. 3, and classes 3 *vs*. 4.

Results

Seedling density

There was a positive association between seedling and adult densities (r_S= 0.42, P< 0.01), hence adult density was used as a covariate in the analysis of variance. In this analysis, seedling density did not show significant differences neither between locations (χ^2= 0.38, d.f.= 2, P= 0.83), nor between fragment-types (χ^2= 2.65, d.f.= 2, P= 0.27). Moreover, although, statistical differences were found in the interaction location x fragment-type (χ^2= 11.41, d.f.= 2, P= 0.01), no differences were found between fragment types K and KE (d.f.= 1, Pajcha: χ^2= 0.77, P= 0.38, Pintumayu: χ^2= 3.31, P= 0.06, Thola P'ujru χ^2= 0.33, P= 0.57) or K and KP at any location (d.f.= 1, Pajcha: χ^2= 3.09, P= 0.08, Pintumayu: χ^2= 0.65, P= 0.42, Thola P'ujru χ^2= 2.22, P= 0.14). The seedling density per 100m^2 plot ranged from six individuals in the fragment type KE in Thola P'ujru to 31 individuals in the small fragment type KP in the same locality; among all plots the mean density was 16, and its standard deviation was 10. The adult density explained significant parts of the variation in seedling density (χ^2= 6.29, d.f.= 1, P= 0.03).

Growth form structure

The growth form structure of *P. subtusalbida* seedlings were significantly different between locations (χ^2= 10.87, d.f.= 4, P= 0.03) but not between fragment types (χ^2= 5.50, d.f.= 4, P= 0.24). The differences found at each location varied among fragment-types (Fig. 5.2, χ^2= 26.26, d.f.= 8, P= 0.01).

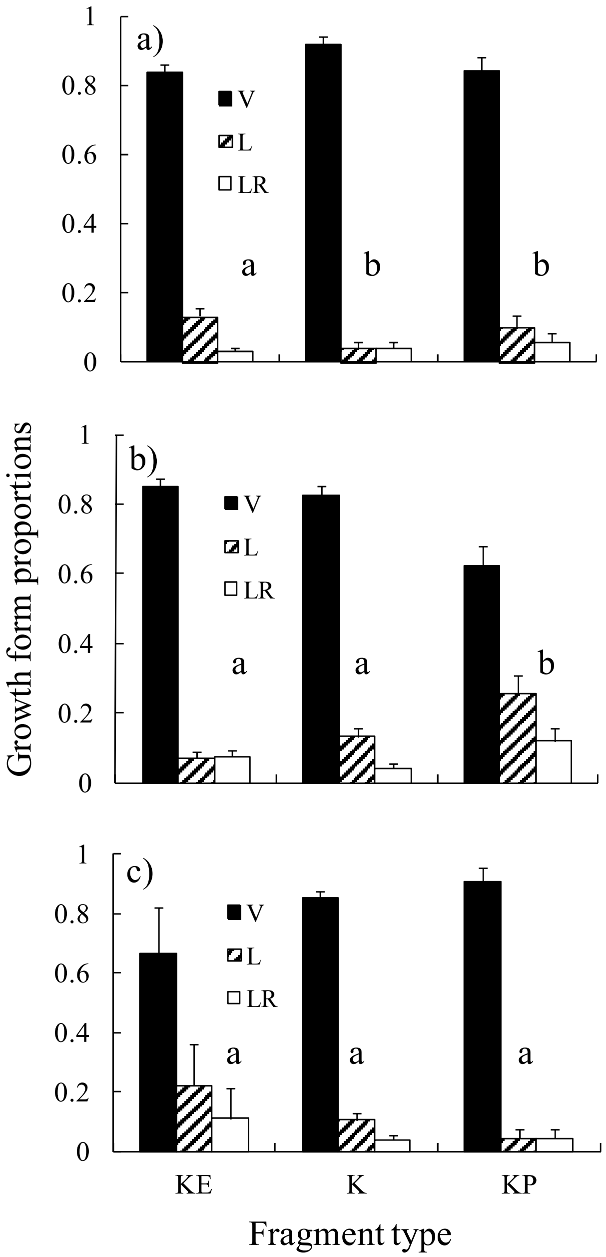

Fig. 5.2 Growth form structure of *Polylepis subtusalbida* seedlings (height between 10 and 140cm) with vertical growth (V), lateral growth (L) and lateral growth with adventitious roots (LR) at fragment-types: *P. subtusalbida-Eucalyptus globulus* (KE), *P. subtusalbida-Pinus radiata* (KP) and *P. subtusalbida* only (K), at three location: (a) Pajcha, (b) Pintumayu and (c) Thola P'ujru. The growth form structures with the same letter are not statistically different ($P \geq 0.05$).

In Pajcha (Fig. 5.2a), the growth form structure was the same between K and KP fragments (χ^2= 3.71, d.f.= 2, P= 0.16), although it was different between K and KE fragments (χ^2= 7.83, d.f.= 2, P= 0.02). In fact, the proportion of individuals with lateral growth (L) was three times larger in KE fragments than in K fragments, while the proportion of individuals with vertical growth (V) was similar between these fragment-types. In Pintumayu (Fig. 5.2b), the growth form structure was not different between KE and K fragments (χ^2= 5.77, d.f.= 2, P= 0.06), but it was different between K and KP fragments (χ^2= 12.27, d.f.= 2, P= 0.002). In fact, the proportion of L individuals was approximately two times lower in K fragments than in KP fragments, and the proportion of seedlings with lateral growth and adventitious roots (LR) was almost three times lower in K fragments than in KP fragments. In Thola P'ujru (Fig. 5.2c), the growth form structure between K and KE fragments (χ^2= 2.14, d.f.= 2, P= 0.34) and K *vs.* KP fragments (χ^2= 1.48, d.f.= 2, P= 0.48) were not different.

Size structure

The size structure was significantly different between locations (Fig. 5.3, χ^2= 18.33, d.f.= 6, P= 0.01) but it was not between fragment types (χ^2= 10.25, d.f.= 6, P= 0.11). The absence of significant differences among fragment types was maintained at each location (χ^2= 19.70, d.f.= 12, P= 0.07). The size structures observed in Pajcha and Pintumayu were not significantly different (χ^2= 5.80, d.f.= 3, P= 0.12), whereas the ones observed in Pintumayu and Thola P'ujru were different (χ^2= 13.01, d.f.= 3, P= 0.005). In fact, the proportion of small individuals (classes 1 and 2) was near to 1.4 times larger in Pintumayu than in Thola P'ujru and the proportion of the largest individuals (class 4) was almost 2.5 times larger in Thola P'ujru than in Pintumayu.

Growth rate

The growth rate of P. subtusalbida seedlings was not significantly different between locations ($F_{2,35.7}$= 2.38, P= 0.11) and between fragment types ($F_{2,40.7}$= 0.41, P=0.67); however, the growth rates found at each fragment-type

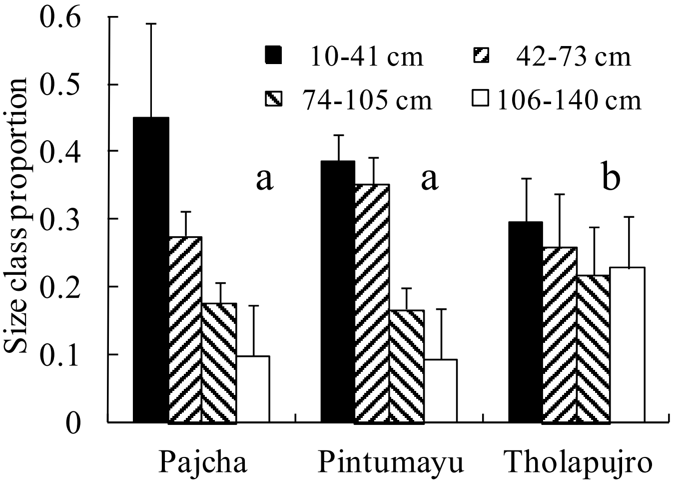

Fig. 5.3 Size structure of *Polylepis subtusalbida* seedlings (height between 10 and 140cm) at three locations: Pajcha, Pintumayu and Thola P'ujru. The size structures with the same letter are not statistically different ($P \geq 0.05$).

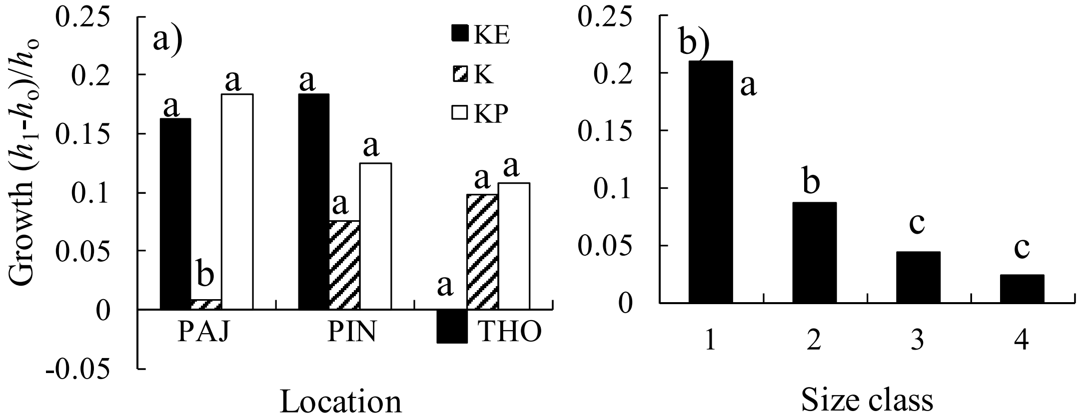

Fig. 5.4 Growth rate of *Polylepis subtusalbida* seedlings (height between 10 and 140cm) where h_0 and h_1 are the height the year 2004 and 2005, respectively. (a) At fragment- types: *P. subtusalbida-Eucalyptus globulus* (KE), *P. subtusalbida-Pinus radiata* (KP) and *P. subtusalbida* only (K), at three locations: Pajcha (PAJ), Pintumayu (PIN) and Thola P'ujru (THO); (b) Growth rate according to size classes. The means with the same letter are not statistically different ($P \geq 0.05$) between fragments within each location (a), and between size classes (b).

were different among locations ($F_{4,31.6}$= 3.75, P= 0.01). In Pajcha, the seedling growth rates in KE and KP fragments were around 20 times larger than that observed in K fragments (Fig. 5.4a, $F_{1,20.1}$= 11.03, P= 0.003 for KE *vs.* K, and $F_{1,26.9}$= 9.94, P= 0.004 for KP *vs.* K). In Pintumayu, the growth rates of seedlings in KE fragments were 2.5 times larger than that in K fragments ($F_{1,22}$= 4.46, P= 0.04), although the growth rates between K and KP fragments were similar ($F_{1,32.1}$= 0.52, P= 0.47). In Thola P'ujru, the growth rates of seedlings were similar among fragment-types ($F_{1,106}$= 2.16, P= 0.14 for K *vs.* KE, and $F_{1,25.1}$= 0.85, P= 0.36 for KP *vs.* K). The growth rates were different between size classes (Fig. 5.4b, $F_{3,936}$= 23.02, P< 0.001). In fact, smaller individuals (class 1) grew 2.4 times faster than the medium-sized seedlings (class 2, $F_{1,933}$= 27.81, P< 0.001). Class 2 seedlings, grew two times faster than the large-sized seedlings ($F_{1,937}$= 4.79, P= 0.03 for classes 2 *vs.* 3, and $F_{1,934}$= 1.58, P= 0.21 for classes 3 *vs.* 4).

Seedling survival

The seedling survival was not significantly different between locations (χ^2= 2.08, d.f.= 2, P= 0.35), fragment-types (χ^2= 1.19, d.f.= 2, P= 0.55), and there was no interaction between location and fragment-types (χ^2= 4.68, d.f.= 4, P= 0.32), neither between growth forms nor fragment-types (χ^2= 0.24, d.f.= 4, P= 0.99). Hence, fragment-types and locations seem to be non-important for seedling survival. Significant differences in seedling survival were detected among growth forms (χ^2= 6.39, d.f.= 2, P= 0.04). In fact, the survival probability for seedlings with vertical growth (V) was 0.84 and the survival probability for seedlings with lateral growth (L) was inferior in 12% (Fig. 5.5a, χ^2= 4.52, d.f.= 1, P= 0.03), while the survivorship of growth forms V and LR (seedlings with lateral growth and adventitious roots fixed to the ground) were not different (χ^2= 2.61, d.f.= 1, P= 0.11). Additionally, the proportion of the surviving seedlings was different between size classes (Fig. 5.5b, χ^2= 10.93, d.f.= 3, P= 0.01), being the smallest seedling proportion (class 1) lesser than the proportion of the medium-sized ones (class 2, χ^2= 5.82, d.f.= 1, P= 0.02). The other size classes, with medium-sized and large individuals, did not show significant differences in their survivorship (classes 2 *vs*. 3: $\chi^2 = 0.93$, d.f.= 1, $P = 0.33$, and classes 3 *vs*. 4: $\chi^2 = 0.97$, d.f.= 1, $P = 0.32$).

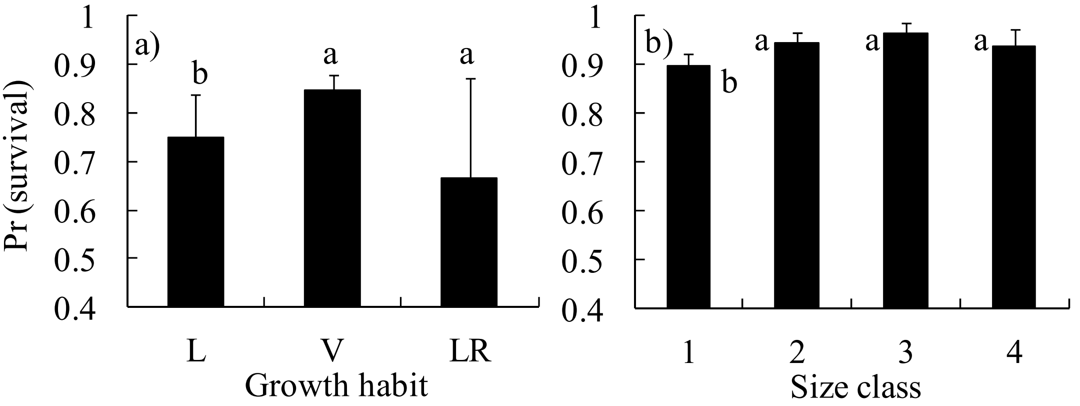

Fig. 5.5 Survivorship of *Polylepis subtusalbida* seedlings (height between 10 and 140cm) according to: (a) growth forms, *i.e.* lateral growth (L), vertical growth (V) and lateral growth with adventitious roots (LR); and (b) size classes. The *bars* correspond to the standard error and means with the same letter are not statistically different ($P \geq 0.05$) between growth form (a) and size class (b).

Discussion

Results of this study may be summarized as follows: (1) seedling density of *P. subtusalbida* did not vary neither among locations, nor among fragment-types; (2) seedlings with lateral growth and with adventitious roots are less frequent in pure fragments of *P. subtusalbida*; (3) seedling size structure did not vary among fragment-types, however it varied among locations; (4) seedling growth was higher in mixed fragments than in pure fragments of *P. subtusalbida*; the smaller seedlings expressed the highest growth rate; (5) seedlings with vertical growth had the highest probability of survival next to seedlings with lateral growth and adventitious roots; the smaller seedlings are the most vulnerable to environmental hazards. Thus, the regeneration pattern of the *P. subtusalbida* at Parque Nacional Tunari is a complex process that at least in some variables ought to be understood at two levels: at fragment and at location level. At some locations (*e.g.* Pajcha) the growth rate of *P.*

subtusalbida seedlings increased when seedlings grew with *P. radiata*, and their survival was only affected by their growth form (the seedlings with lateral growth presented lower survival than the others). At other locations (*e.g.* Pajcha and Pintumayu), the seedlings modified their growth forms (larger numbers of seedlings with lateral growth) in the mixed fragment types. Hence, the hypothesis was rejected for *P. subtusalbida* density, size structure, plant growth and survival, and their interpretation is as follows:

Seedling density, growth forms and survival

Seedling density is more related to adult density than to fragment type; thus, it was not reduced by change in fragment type as reported previously (Larrazabal 2004). Changes in the growth form structure are evidence that *P. subtusalbida* seedlings have the ability to modify their growth form toward lateral forms and may develop adventitious roots more frequently, when they grow with *P. radiata* and *E. globulus* than when they grow in pure *P. subtusalbida* fragments. It could be a way to ‘‘explore’’ the environment searching for limiting resources, for instance, light or nutrients (Fagan & Peart 2004). The ability to modify the growth form, as it was observed in *P. subtusalbida* seedlings, is considered an adaptation of tree species in shady environments (Givnish 1978, cited in Crawley 1997). Growth form was the only variable which supported our hypothesis, *i.e.* the exotic species reduced vertical seedling frequencies.

Over the year of study, changes in survival of *P. subtusalbida* seedlings were related to growth form and size (Fig. 5.5), but they were not directly related to the exotic species presence. The lowest survival of seedlings with lateral growth form and without adventitious roots may be a cause of the etiolation process, which often causes the death of individuals (Hutchings 1997). In fact, field observations of dead seedlings with lateral growth showed no signs of herbivory and remained desiccated but with the leaves and shoot intact. The higher survivorship of seedlings with lateral growth and with adventitious roots would be associated to the fact that adventitious roots offer a larger absorption area (*i.e.* for nutrients and resources) and a greater fixation to the ground. The ability to develop adventitious roots may be considered an adaptation to surmount stressed environments. The high standard errors observed in the seedlings with lateral growth and with adventitious roots (LR) might be caused by the low sample size for this growth habit (n = 15) compared with the others (n vertical = 966, n lateral = 82). Although, the exotic species did not reduce *P. subtusalbida* survival directly, it is plausible to think that this reduction is mediated through the changes in growth form structure. As we saw before, exotic species increased the frequency of lateral growth forms, which in turn have the lowest survival.

Size structure

Exotic species did not affect size structure. The change observed between locations in size structure (lower frequency of seedlings smaller than 41cm height in Thola P'ujru), may be partially due to human activities that seem to be minor in Pajcha and Pintumayu than in Thola P'ujru. In fact, the fragments studied in Thola P'ujru were close to the village, and therefore, could have been more affected by sheep and fires in the past, which, in turn could change their size structure, decreasing seedling recruitment, and reducing the frequency of small seedlings. It is recognized that human activity such as fire, sheepherding, and trampling affects native plant populations negatively (Renison *et al.* 2002; Aragón & Morales 2003; Renison *et al.* 2005), a combination of these activities could have contributed to the generation of observed patterns.

Growth rate

The growth of *P. subtusalbida* seedlings was greater when they coexisted with *P. radiata* and *E. globulus* than when they were growing alone (Fig. 4a). Greater growth of seedlings does not necessarily imply a facilitation effect from exotic to native plants (Walker & Vitousek 1991; Callaway & Walker 1997). In this case (see seedling density and size structure), it is more plausible to think that greater growth of seedlings was a result of competition for light. Due to competition, *P. subtusalbida* seedlings preferred to invest growth in height (Givnish 1982, cited in Crawley 1997) through internode expansion to reach light resources (etiolation, Hutchings 1997).

The absence of changes in density, growth form structure, growth rate and survival of *P. subtusalbida*'s seedlings at some locations and fragment-types, are evidences that these seedlings are able to coexist with the exotic trees. Probably, this coexistence occurs under low plant densities or low canopy coverage of these exotic trees. Another important point is that the exotic species were likely planted on sites that land managers believed the species would be successful, and these sites were chosen based on logistics, practicality, and need for erosion control; therefore the differences between mixed fragments and native fragments can also be interpreted as locational effects. However, no data about abiotic environmental conditions were collected in the studied fragments. For these reasons, further investigations are needed to examine the competition between these native and exotic trees. Specifically, appropriate experiments either at the field or in the greenhouse should be designed in order to discriminate whether the competition happens at canopy or roots level (Gibson 2002), or if the effect of the exotic species is related to their density, height, coverage, or age. This should be done in such a way, that the competition, its magnitude, and possible mechanism might be determined.

It would also be important to decipher which kind of competition is more important (inter or intra-specific competition; exploitation or interference competition). That is, auto-ecological, population, or community ecology studies involving native and exotic trees are urgent tasks to initiate at the Parque Nacional Tunari (PNT). Moreover, field experiments dealing with seed dispersal, seed bank dynamics, germination, and seedling establishment are important to elucidate critical phases of the *P. subtusalbida* life cycle. Additionally, incorporating these studies in the different fragment-types that exist at the landscape of PNT, would let us know how *P. radiata* and *E. globulus* influence these processes. Another urgent task is to elucidate whether *P. radiata* and *E. globulus* are really invaders, and whether they are producing significant ecological impacts as it has been documented at other regions of the world (Richardson & Cowling 1994; Richardson 1998; Le Maitre *et al.* 2002; Bustamante & Simonetti 2005).

The primary reason to introduce exotic species in Bolivia was to control erosion (FAO 2003). At present, these species may be affecting the native tree species regeneration, such as *P. subtusalbida* at the Parque Nacional Tunari (PNT). The variation in regeneration patterns observed among locations and among fragment-types, suggests that any measure for sustainable management of these forests should be contingent to specific locations. General practices such as pruning and extraction of *P. radiata* and *E. globulus* seedlings close to young and adult *P. subtusalbida*s (not necessarily in fragments) ought to be considered. The maintenance of *P. radiata* and *E. globulus* at PNT has to be considered, taking into account their system functions (prevention of flooding and erosion), and if other species may accomplish these. In summary, to decide the future of these exotic trees it is necessary to consider risks, costs, and benefits (Richardson 1998) of exotic species maintenance at the Parque Nacional Tunari.

CHAPTER 6

Regional climate *vs*. radial growth relationships for *P. besseri*

Adapted from Gareca, E.E., M. Fernández, & S. Stanton. 2010. Dendrochronological investigation of the high Andean tree species *Polylepis besseri* and implications for management and conservation. *Biodiversity and Conservation* 19: 1839-1851.
The original publication is available at www.springerlink.com

Introduction

Management and conservation of tropical forests benefit from a better understanding of the relationship between tree growth and climate variables (Vetter & Botosso 1989). This information is crucial for efficient forestry management of plantations and native woodlands (Jacoby 1989; Koslowski & Pallardy 1997), and increases our overall understanding of how tropical forests function (Mares 1986; Ehrlich & Wilson 1991; Gentry 1992).

Research in Bolivian forests has focused on estimating ages and growth rates of commercial tree species such as *Bertholletia excelsa* (Lecythidaceae) and *Cedrela odorata* (Meliaceae) (Brienen & Zuidema 2006). Such information is limited for less economically important species such as *Polylepis besseri* Hieron. (Rosaceae). In the mountain range of the Tropical and Subtropical Andes, *Polylepis* is the only genus with a tree life-form that can grow at high altitudes. This genus is distributed from Venezuela (9°N) to Argentina (32°S) at elevations ranging from 1800m a.s.l., in Córdoba, Argentina, to 5200m a.s.l., in Parque Nacional Sajama, Bolivia (Simpson 1993). Recent research has examined the relationship between climate and growth for the Andean tree *Polylepis* growing in two out of the four Andean biogeographic provinces of Bolivia (Navarro & Maldonado 2002): the dry and cold Altiplanic Province (Argollo *et al.* 2006) and in the humid, pluviseasonal and cold Perú-Bolivian Yungas Province (Roig *et al.* 2001). In contrast, little is known about patterns in growth and climate response for *Polylepis* in the sub-humid, pluviseasonal and cold Peruvian Puna Province.

Currently there is a lack of research related to the growth rate estimation of *P. besseri*, which would be useful for development of sustainable management strategies for the woodlands avoiding possible local extinction (World Conservation Monitoring Centre 1998a, see Chapter 2). The identification of the climatic factors influencing growth would help conservation efforts, allowing managers to locate the reforestation with *P. besseri* in more suitable places in order to increase the growth rate and regeneration of the *Polylepis* woodlands.

We studied annual ring formation, growth rates, and climatic response of *P. besseri* in the Peruvian Puna Province of Bolivia. The relationship between *Polylepis* growth and climate remains poorly understood in this biogeographical Province. Our specific objectives were to: 1) determine if *P. besseri* is useful for dendrochronological research, 2) estimate growth rates of two *P. besseri* woodlands, and 3) determine the main climatic factors influencing *P. besseri*'s radial growth.

Materials and Methods

Species and study site

Polylepis besseri Hieron. in Sacha Loma, Cochabamba-Bolivia was already described (see Chapter 3). *P. besseri* trees at this site (Fig. 6.1) experience litter fall around the year with a peak in May and June. Shoot growth is from November until August, peaking in February and March (Martinez-Costas 2003).

Two *P. besseri* woodlands were selected from a sample of seven woodlands used for fragmentation studies by the Centro de Biodiversidad y Genética in Cochabamba (Fig. 6.1). The study site is located in Sacha Loma, Cochabamba (17º44'S, 65º34'W, Fig. 6.1). Altitudinal range of the fragment in mountain Uypa (fragment Uypa, 34.9ha) is 3600-3900m and 3500-3850m for the fragment on mountain Chutu Senega (fragment Chutu Senega, 47ha). Slope angles vary from 9º to 32º for the fragment Uypa and from 1º to 38º for the fragment Chutu Senega. The study fragments are separated by a linear distance of 1318m.

Mean annual temperature of the region is 8.7ºC and total annual precipitation is 721mm (Fernández *et al.* 2001). The humid and warm growing season occurs from November to March; the dry and cold season is from June to August with minimum temperatures as low as -5.2ºC in July. According to the bioclimatic model of Rivas–Martínez *et al*. (1999), the study area has a pluviseasonal bioclimate and is in the superior supratropical, humid inferior floor (Fernández *et al.* 2001).

Fragment Uypa includes both open and closed *Polylepis* woodlands. The tree canopy consists exclusively of *P. besseri*, averaging 300cm in height with monopodic and sympodic ramification of the trunks. The understory vegetation is dominated by *Berberis* (Berberidaceae), *Ribes* (Grossulariaceae) and *Gynoxys* (Asteraceae) between 50-250cm in height and the grasses of the genus *Festuca*, *Stipa* and *Calamagrostis*, which reach 60cm in height. The higher parts of the fragment Uypa have *P. besseri* trees with a mean diameter of 8cm and a mean height of 250cm. Above this woodland (3900m), there is grassland between 5-15cm in height, which has different species of forbs, graminoids, cacti and bromeliads, which constitute the matrix vegetation of the Peru-Bolivian Andean Puna.

Fragment Chutu Senega consists of mainly open woodland with some areas of dense vegetation. The tree canopy includes only *P. besseri* trees of similar size to those in the fragment Uypa. The understory has the same shrubs and herbaceous species as Uypa, but with the addition of the pyrophilous species *Orthrosanthus chimboracensis* (Iridaceae), *Gentianella* aff. *incurva* (Gentianaceae) and the nitrophilous *Caiophora canarinoides* (Loasaceae). In some higher zones of the fragment Chutu Senega (3850m), there are *P. besseri* trees with mean diameter of 25cm and 400cm in height, but these areas were not sampled because we limited our sampling to one ridge.

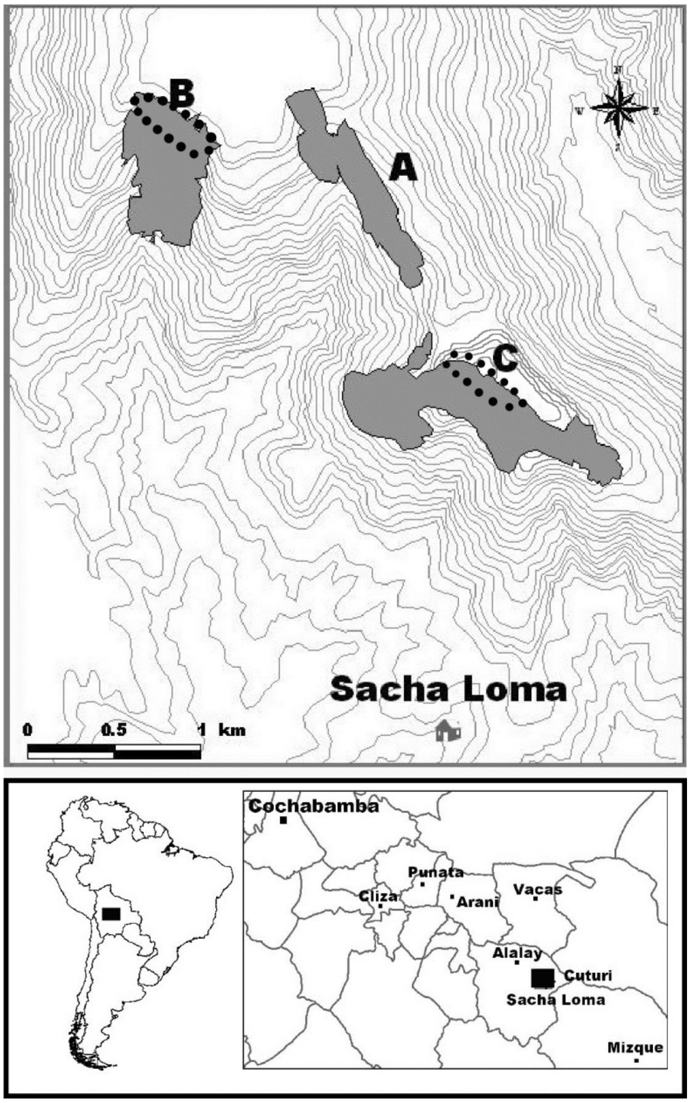

Fig. 6.1 Study area. *Gray surfaces* are the *Polylepis* fragments, *dotted areas* represent the sampling sites, and *letters* (A, B and C) represent fragment codes given by the Centro de Biodiversidad y Genética. B = Uypa fragment, C = Chutu Senega fragment. After Cahill & Matthysen (2007).

Tree selection and sampling

The sampling was limited to the higher parts of the fragments to ensure sampling trees with greater climate sensitivity. We sampled canopy dominant trees growing in relatively flat places, with wide trunks and symmetrical canopy growth. Multiple stemmed trees with fire scars or other injuries were avoided. We obtained complete cross-sections of the trunks approximately 50cm above the ground. In Uypa, we collected only five sections near the higher limit of the fragment (3900m), which we combined with 31 previously sampled disks from a collection made in 1999 by Mendoza (2001) in the same site and with the same criteria reported above. For Chutu Senega, all 25 cross-sections were obtained from trees near the higher limit of the fragment (3800–3850m). We sampled in March and April of 2001, and we prepared samples following standard dendrochronological techniques (Roig *et al.* 2001).

Crossdating, growth and chronologies for *P. besseri*

We measured two series of tree-rings from each cross-section to the nearest 0.001mm using a Velmex measuring system coupled to a magnifying device. The samples from each fragment were crossdated independently. First, the two series per tree were crossdated visually using narrow and wide tree-rings. Then we generated graphs for each series using the list method (Yamaguchi 1991) and the routine BAR from DPL (Holmes 1999a). Thereafter, and with the shortest series with well delimited tree-rings, a graphic evaluation of the synchrony between pairs of series was made until a graphic synchrony was obtained among five or six trees per fragment. Additional series were then added one at a time and checked for synchrony and possible errors using the software COFECHA (Holmes 1999b). Ring-widths were re-measured as necessary.

We compared tree ages between fragments using the non-parametric Mann-Whitney U-test with adjustment for ties using STATISTICA (StatSoft Inc. 2001). The relationships between diameter and age were carried for the trees of both fragments using non-parametric Spearman correlations. Master chronologies were obtained for each fragment using the crossdated series and the software ARSTAN (Cook & Holmes 1999). Trends due to growth were removed using a cubic spline filter retaining 50% of the variance for periods of 10yr. This detrending was chosen to remove low frequency variance (*e.g.* due to growth) and to enhance the inter-annual variation related to climate variables. In other words, detrending and standardizing ring-width data provides a growth index that does not depend on the growth period of the tree and that contains the highest climatic signal (Cook & Holmes 1999). For comparing the average tree ring width between fragments, we performed a paired t-test of standardized ring width indexes.

Relationship between climate variables and radial growth

We used mean temperature and monthly precipitation data (Fig. 6.2) from the nearest meteorological station of Arani (17°34'S, 65°46'W, 27km from the study site, and at 2865m). The precipitation record is since 1960 (40yr) and the temperature since 1970 (30yr). We calculated Spearman rank correlations to identify the relationship between the climatic data and the *P. besseri* growth data. Specific climate variables included were monthly precipitation, and minimum, average and maximum temperature, for November to March of the current year of growth and for each month of the previous year of growth. We performed a second analysis summarizing the information in all the climatic variables during their common period (since 1970, 30yr) into principal components using procedure PRINCOMP in SAS v. 9.2 (SAS Institute Inc. 2008). Then, we calculated Spearman rank correlations with these new summarized climatic data and *P. besseri* growth data to identify the relationship between growth and climate. Finally, and to look for evidence of El Niño Southern Oscillation (ENSO), which is an ocean-atmosphere phenomenon with irregular fluctuations between warm (El Niño) and cold (La Niña) phases, and has indirect effects over much of South America (Garreaud *et al.* 2009), we used the sea surface temperature (SST) anomalies (Niño 3.4 index) from the Niño region 3.4 (Trenberth & Stepaniak 2001) available at www.cgd.ucar.edu/cas. We performed the same analysis used for the climatic data (*i.e.* spearman correlations between the growth data from both fragments and the raw data and principal components of the SST anomalies).

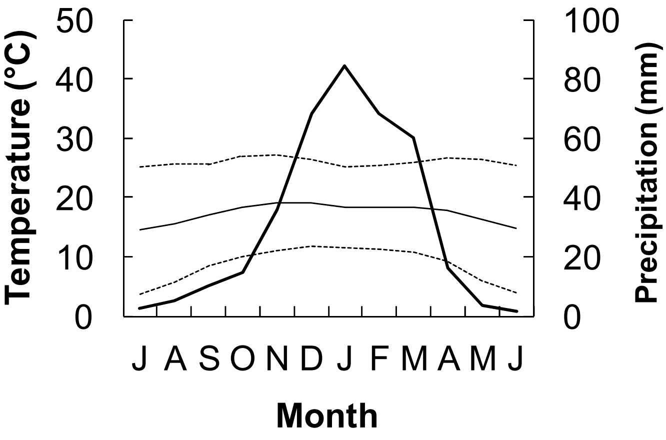

Fig. 6.2 Climate diagram of Arani with monthly precipitation (mm, *weighted line*), maximum, minimum (*dotted lines*) and mean temperature (ºC, *solid line*).

Results

Annual ring formation and tree ages

Anatomically, the wood of *P. besseri* exhibited semicircular porosity. The differences in diameter between the early- and latewood cells, as well as the presence of fibers with thick cell walls and 'radially' flat in the latewood, allowed us to differentiate annual growth rings (Fig. 6.3). The majority of each cross-section was heartwood, where tree-ring differentiation was easier compared to the sapwood.

We successfully crossdated 18 series from 11 trees from Uypa, and 21 series from 12 trees from Chutu Senega (Table 6.1) indicating that the error in the age estimation per tree in each fragment is minimal and allowed us to compare the ages between both fragments. Mann-Whitney tests indicate the ages of the trees were significantly different (U= 24, Zadj= 2.59, *P*= 0.01), with trees in Chutu Senega being on average 10 years older than those in Uypa (Table 6.1). No relationship was found between the diameter and age for the trees of both fragments (Uypa n= 11, r_s= 0.16, *P*= 0.65, Chutu Senega r_s= -0.15, *P*= 0.65). Our intentions to crossdate the tree-ring widths from both fragments in a single chronology failed; thus, radial growth from both fragments was analyzed independently. .

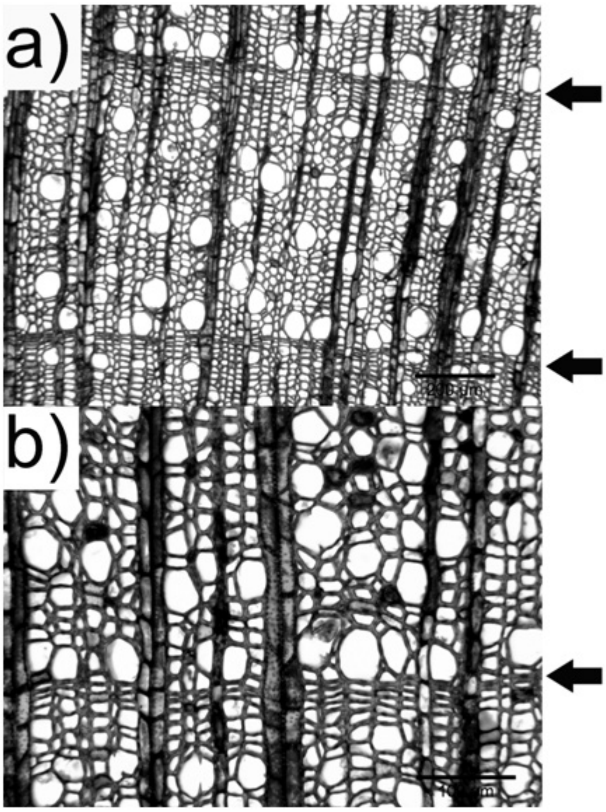

Fig. 6.3 Tree-rings of *Polylepis besseri* a) a complete tree-ring can be distinguished between both arrows. The *line* represents 200µm. b) A detail of the tree-ring (*arrow*).The line is 100µm.

Chronology development and growth

Ring-width chronologies were developed independently for each woodland fragment, spanning 60yr for Uypa and 63yr for Chutu Senega (Fig. 6.5). The mean correlations between trees and the mean sensitivity were similar for both fragments (Table 6.1). The higher signal/noise relation (Table 6.1) for the detrended series from Uypa compared with Chutu Senega indicates that the Uypa trees were more sensitive to yearly environmental variation. Additionally, the average tree ring width was larger in Uypa than in Chutu Senega trees (n= 60, t= 4.54, *P*= 0.01, Table 6.1 and Figs. 6. 4 and 6.5).

Table 6.1 Summary statistics for two fragments of *Polylepis besseri* (Sacha Loma–Bolivia).

Statistics	Uypa	Chutu Senega
Sampled trees	36	25
Cross dated Series/Trees[a]	18/11	21/12
Master series (yr)	1941-2000 (60)	1938-2000 (63)
Crossdated rings[a]	668	1019
Series intercorrelation[a]	0.452 (P=0.01)	0.390 (P=0.01)
Mean sensitivity[a]	0.405	0.418
Autocorrelation[a]	0.411	0.342
Mean / median age (yr)[b]	39.7 / 34	52.7 / 51.5
Mean tree-ring (mm)[c]	0.74	0.58
COMMON INTERVAL[d]	1973-1998 (26)	1956-1998 (33)
Signal/Noise relation	3.387	1.932

[a] Values obtained with COFECHA (Holmes, 1999b).
[b] Values statistically different using a Mann Whitney test (*P*= 0.01).
[c] Values statistically different using a paired t-test (*P*= 0.01).
[d] Values obtained with ARSTAN (Cook and Holmes, 1999).

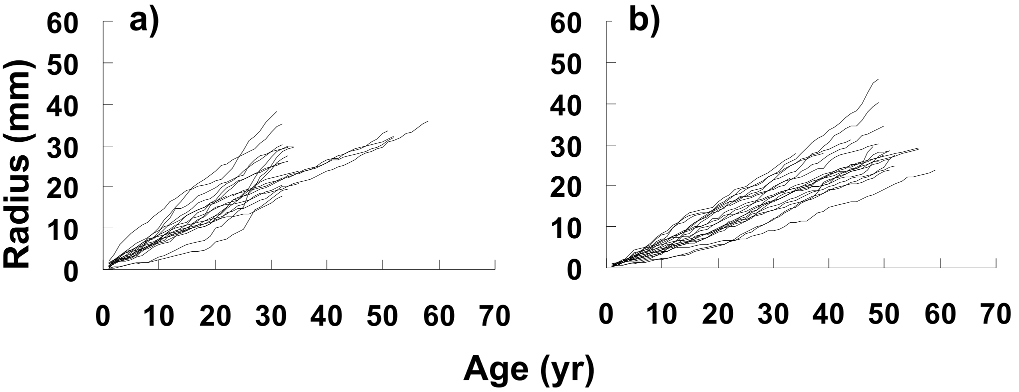

Fig. 6.4 Cumulative radial increments (mm) of *P. besseri* per series for fragments (a) Uypa (n=18) and (b) Chutu Senega (n=21).

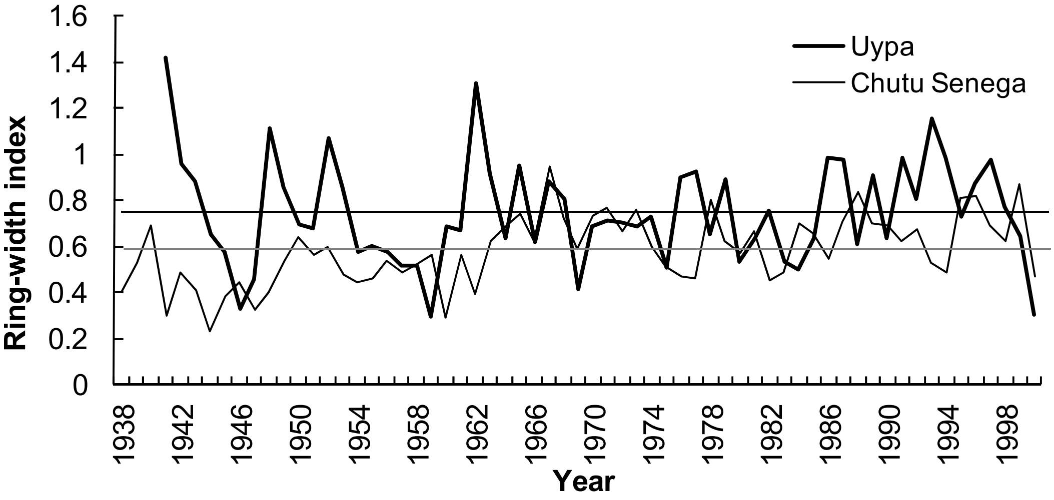

Fig. 6.5 Ring-width chronologies for *Polylepis besseri* at Sacha Loma.

Relationship between climate variables and tree growth

The climate data from Arani showed significant associations with radial growth of *P. besseri* from both fragments. Trees from Chutu Senega invested more in wood production when precipitation was low at the beginning of the humid period of the previous growth season (November, Fig. 6.6). Trees from Uypa showed an increase in growth when the previous winter was drier than average (June, Fig. 6.6).

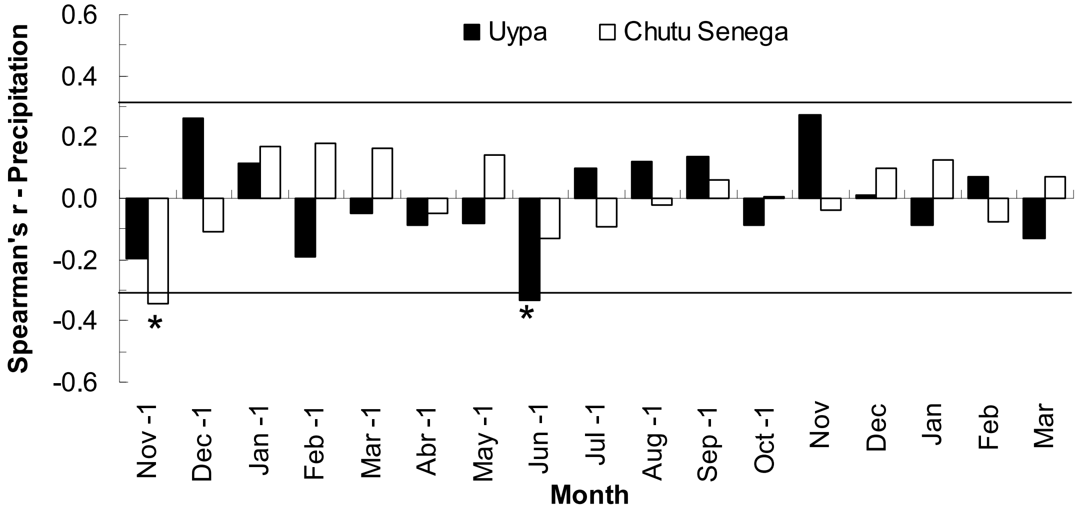

Fig. 6.6 Estimated correlation coefficients between the ring-width chronologies of *Polylepis besseri* for fragments Uypa – Chutu Senega and total precipitation recorded at Arani. Horizontal lines represent 95% confidence intervals; * indicates significant effects ($P< 0.05$).

There were three significant relationships between tree growth and temperature data. The average or maximum temperature, before and during the growing season (October and January), had a positive effect on the ring widths of Uypa (Fig. 6.7), indicating that warmer periods improved annual growth. No significant relationship between diameter growth and temperature was found for trees in Chutu Senega (Fig. 6.7).

The climatic data were summarized in two principal components (PC1 and PC2) which together explained 33% of the total variation. The correlations between these climatic dataset and radial growth of *P. besseri* were no-significant (PC1: Uypa $r_S = -0.04$, $P= 0.81$, Chutu Senaga $r_S = -0.15$, $P= 0.42$;

PC2: Uypa $r_S = 0.27$, $P = 0.14$, Chutu Senaga $r_S = -0.33$, $P = 0.07$). Regarding the effects of ENSO on growth, both analyses (*i.e.* using the raw data and using the principal components for the SST anomalies) showed a positive and a negative association between SST anomalies and growth for Uypa and Chutu Senega trees, respectively. The first principal component for SST anomalies, which explained 61% of the variation in the data, was positively correlated with *P. besseri* tree growth at Uypa ($r_S = 0.32$, $P = 0.01$) and negatively correlated with tree growth at Chutu Senega ($r_S = -0.31$, $P = 0.02$).

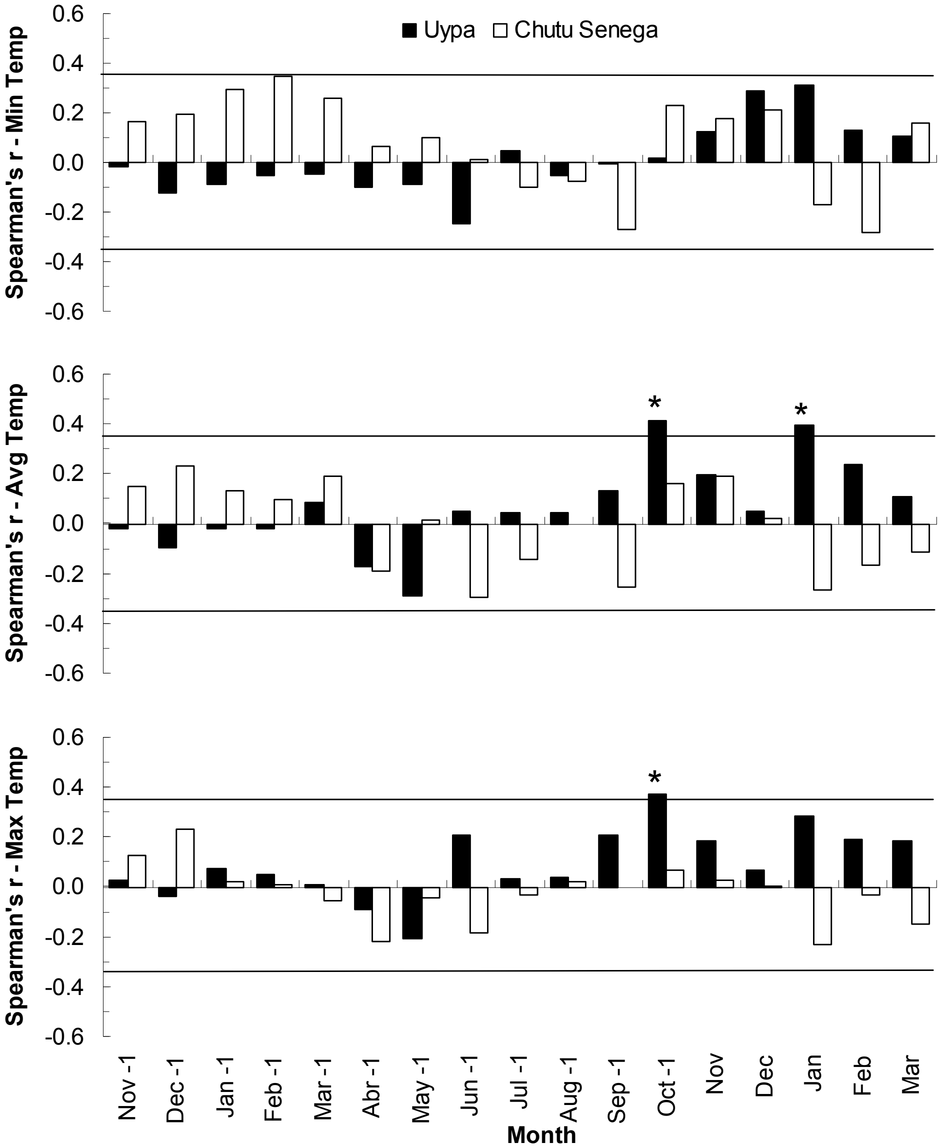

Fig. 6.7 Estimated correlation coefficients between the ring-width chronologies of *Polylepis besseri* for fragments Uypa – Chutu Senega and the mean monthly temperature recorded at Arani. *Horizontal lines* represent 95% confidence intervals; * indicates significant effects ($P < 0.05$).

Discussion

Annual ring formation

Our experience indicates that *P. besseri* generates annual rings that are suitable for assessing age, radial growth rate, and relationships with climate. It was difficult to identify annual rings in increment core samples, but a good view of them was possible using disk samples and 40x amplification. The heartwood rings, characterized by the accumulation of pigments, were more distinguishable than those in the sapwood.

The pattern found in *P. besseri* rings was similar to that described for a sister taxa *P. pepei*, but the latter showed a circular tendency in the conductive vessels, a characteristic that makes it easier to work with increment core samples (Roig *et al.* 2001). Future research on *P. besseri* would benefit from less destructive sampling methods that would allow for more thorough investigations without further endangering the woodlands. Our samples showed no significant correlation between age and diameter, therefore wood samples are necessary for estimating tree age. The small diameters of *P. besseri* trees makes them good candidates for identifying annual rings with thin anatomical sections taken from increment cores, but more research is needed to verify this possibility.

Crossdating and tree ages

Crossdating was moderately successful within each fragment, but we were unable to crossdate both fragments together. Chutu Senega trees were 10yr older on average and had higher mean sensitivity compared with those from Uypa. This may explain why a larger proportion of the Chutu Senega samples crossdated well. We sampled the largest trees in the high areas of both fragments; therefore our results indicate that the higher part of Uypa was more recently colonized than the high elevations in Chutu Senega or that the oldest trees from Uypa were recently logged.

Our results also suggest that 60yr is relatively young for *P. besseri*, as indicated by growth curves that have not leveled off as is typical for mature trees. The maximum age (63yr) found among the sample trees in the two fragments studied was less than those reported for *P. pepei* (132yr, Roig *et al.* 2001) or *P. tarapacana* (705yr, Argollo *et al.* 2006).

Chronology development and growth

The high mean sensitivity, moderate series intercorrelations, and anatomical features of the tree rings indicate that *P. besseri* forms annual rings useful for dendrochronological research. This is the first time, to our knowledge, that *P. besseri* growth rates and lifespan have been estimated.

The average radial growth was relatively slow (<1mm/yr) in both sampled woodlands. The trees were growing slowly, but steadily at least for the first 60yr in both fragments. The Uypa trees were younger and growing faster than Chutu Senega trees, and showed different temporal patterns in growth (Fig. 6.5). Uypa trees did not experience the growth declines observed in Chutu Senega during the years 1962, 1976-1977, 1986, and 1994; in fact, Uypa trees grew well during those years. These results suggest the fragments may have different limiting factors for growth. Soil depth and exposure are similar in both fragments, but other local factors may improve tree growth in Uypa. Two of these can be local disturbance and nutrient availability. The herb layer at Chutu Senega included pyrophilous species, meaning that fires are important in this fragment. Nitrophilous species were also found in Chutu Senega, their presence might be due to the presence of herbivores and therefore an addition of nitrogen to the soil as well as grazing may occur in this fragment, thus anthropogenic disturbance may affect Chutu Senega. Since the fire history of the place is unknown and studies are being conducted about the human disturbances in this area, more research is needed to test the local disturbance and nutrient availability hypotheses. A third important factor might be climate. This is the main regional factor, therefore, it is possible Uypa and Chutu Senega trees respond in a different way to the regional climate conditions or experience different microclimates.

Relationship between climate variables and tree growth

The relationships between radial growth and climate summarized as principal components were no significant. Thus, the discussion below is referred to the relationships between growth and the raw climatic variables. Relationships between radial growth and precipitation were weak, despite the high seasonality of precipitation in this region. There was a weak but significant negative relation with precipitation early in the growing season of the previous year at Chutu Senega. In this fragment, moist conditions in November of the previous growing season may result in more understory growth that increases inter-specific competition with the *P. besseri* trees or it may be that the rain available in this month results in more energy allocation into fruit production compared to years with low rain. The latter hypothesis could be tested by studying the inter-annual variation in fruit abundance and how it relates to precipitation. The negative relationship between growth and precipitation of the previous dry season (June) for trees growing at Uypa may be explained by a reduction in inter-specific competition caused by a delay in the emergence of the herb and shrub layers.

Temperature appears to be a more useful variable than precipitation for predicting radial growth of *P. besseri* at Uypa, maybe because the sampling was done at high elevations, where temperatures are more likely to be the limiting factors (Schweingruber *et al.* 1989). Years with high temperature (average and maximum) before the rainy season (October) produced a higher radial growth in the trees of this fragment. The average temperature in the middle of the growing season also played a role in determining the growth of the trees, since years with a higher temperature than usual in January increased the tree-ring width at Uypa.

Some differences between the two study sites were observed regarding their response to climate. Tree-ring growth of both fragments depends on the precipitation the year before the growing season, but only Uypa trees responded to temperature. These differences, despite that the trees are in close proximity and under ecologically similar conditions, suggest that local factors modify the effects of the regional climate factors on growth of *P. besseri*.

Regarding the effects of ENSO on the radial growth of *P. besseri*, the warm anomalies associated with El Niño events tend to increase growth, while the negative-cold anomalies associated with La Niña events tend to decrease growth at Uypa. At Chutu Senega, the relationships were the opposite, warm anomalies decreased growth, while cold anomalies increased growth. These results were unexpected because of the few correlations found between the climate from the nearest meteorological station (Arani, 27km away the study site) and the tree growth. The opposite response of the trees to the SST anomalies can be explained if we assume that Uypa trees have an additional water source compared to Chutu Senega. This source of water can be fog that ascends more at Uypa and that can be used by *Polylepis* (Yallico 1992) or a higher water level at Uypa compared to Chutu Senega. Under this assumption, an increase in temperature, which is the result from El Niño event in South America (Garreaud *et al*. 2009), would increase growth at Uypa because of the extra water available, whereas the same increase in temperature would reduce growth at Chutu Senega because of the reduction in the soil water availability caused by the warmer temperatures.

It appears that the Uypa site is more suitable for *P. besseri* growth because the trees are younger and with a higher radial growth than the ones in Chutu Senega. As stated before, the study sites are similar in altitude, slope aspect and angle, therefore topographic position does not explain the differences in growing conditions. Intra-specific competition is also unlikely to explain the differences in habitat because tree densities in the higher parts of the fragments are similar, although below ground characteristics of the trees were not measured. Therefore, the difference observed must be related to other abiotic, biotic and anthropogenic factors.

Previous research reports that *Polylepis* can use fog as a water resource (Yallico 1992) and we observed more fog ascending in Uypa than in Chutu Senega. This possibility can be investigated by collecting weather data at each site, rather than relying on regional weather station data. Also, there may be genetic differences in the populations that make trees in Uypa better adapted to the conditions than the ones in Chutu Senega. This can be studied with transplantation experiments and comparisons of genetic diversity. Finally, anthropogenic disturbance could be degrading the Chutu Senega habitat. Chutu Senega is close to the local village Sacha Loma, it has more trees showing evidence of fire (Fernández *et al.* 2007), and pyrophilous and nitrophilous herbaceous communities are more frequently found in Chutu Senega rather than in Uypa, suggesting the presence of fire and large herbivores in the Chutu Senega site. Grazing and pruning for fuel may also occur mainly in this fragment, but more research is needed regarding the anthropogenic disturbances present in these *Polylepis* fragments.

This research demonstrates the potential application of dendrochronology for studying abiotic, biotic and anthropogenic effects on *Polylepis* woodland dynamics at different scales in places lacking long term historical data. While our conclusions are limited by the small sample size, this study adds to the scarce but increasing knowledge of the ecology and dynamics of these endangered ecosystems. Our results provide a foundation for future research of resource allocation in *P. besseri*, inter-specific competition, response to regional and local climate factors, population dynamics and genetic diversity, as well as the effects of anthropogenic disturbances.

The low annual growth rate of *P. besseri* should be considered in the development of long term management plans and also for environmental education of the local communities, which overestimate tree growth (Hagaman 2006). Although the results are not consistent, they suggest the climate in the months prior to the growing season is the most important for tree growth, but more research is needed to explain the mechanisms behind this relationship. The indications that local factors are more important than regional factors for these trees in the studied fragments suggest that *P. besseri* are not ideal subjects for dendrochronological studies, it also makes it difficult to recommend possible locations for reforestations using regional approaches, but it might be an advantage for conservation efforts because local factors are easier to modify than regional factors. The threatened status of the species (World Conservation Monitoring Centre 1998a, see Chapter 2), along with the low growth rates, highlights the need for direct studies and actions towards the conservation of *P. besseri*. Combining our results with information about regeneration patterns will improve efforts to conserve *P. besseri* woodlands and educate local communities about sustainable use.

CHAPTER 7

The population genetics of fragmented *Polylepis* woodlands

Adapted from Gareca, E.E., P. Breyne, K. Vandepitte, J.R.A. Cahill, M. Fernández, & O. Honnay. Genetic diversity of the Andean *Polylepis* woodlands and inferences regarding their fragmentation history. *Conservation Genetics* (submitted).

Introduction

Most landscapes above the timber line of the Andes in South America are dominated by monotonous bunchgrass vegetation. However, a handful of pristine timberline areas consist of woodland islands dominated by monospecific stands of the arborescent genus *Polylepis* Ruiz & Pav. (Rosaceae). The genus consists of 15-30 species (Schmidt-Lebuhn *et al.* 2010) and occurs from Venezuela in the North (9°N) to Argentina in the South (32°S) (Fjeldså & Kessler 1996). The species result from one of the most important Andean radiations and likely evolved during the uplift of the Andes, colonizing new niches as they became available in the Plio-Pleistocene (Schmidt-Lebuhn *et al.* 2010). *Polylepis* woodlands support high biodiversity (Gareca *et al.* 2010b, see Chapter 2) and provide important ecosystems services related to erosion control, water regulation and firewood production (Fjeldså & Kessler 1996).

Determining the timing of the main fragmentation of *Polylepis* woodlands (see Chapter 1) is very important when evaluating conservation strategies. *Polylepis* woodlands are currently considered as being among the most threatened ecosystems in South America (Herzog *et al.* 2002). They will have to cope with unprecedented rates of climate change in the high Andes

(Bush *et al.* 2004), and little is known about their potential response (Gareca *et al.* 2010b, see Chapter 2). Their main threats are the increasing anthropogenic influence related to overgrazing, firewood extraction, burning to maintain adjacent pastures, overcutting because of increased accessibility due to road building and mining (Fjeldså & Kessler 1996; Purcell & Brelsford 2004). If the scattered distribution of *Polylepis* woodlands is of recent anthropogenic origin, the potential of large areas of the high Andes to support woodlands with native species presents an immense challenge for ecosystem conservation and restoration (Kessler 1998), and offers opportunities regarding carbon sequestration projects in the high Andes.

Because habitat fragmentation has well known effects on the population genetics of plant species, insight in the distribution of genetic variation within and among fragmented *Polylepis* woodlands can help to resolve the question regarding the timing of the fragmentation. Habitat fragmentation reduces population sizes and also hampers gene flow between fragments (Young *et al.* 1996). Small populations are more susceptible than large populations to the loss of alleles through random genetic drift (Wright 1931). When lost alleles are not replenished through gene flow, genetic variance will be lost each generation at a rate that is inversely related to the effective population size. As a consequence, the amount of genetic variation held within small fragmented populations will decrease, and the degree of genetic differentiation among populations will increase, until a new equilibrium between gene flow and drift is reached (Young *et al.* 1996; Honnay & Jacquemyn 2007; Aguilar *et al.* 2008). Given that the effect of genetic drift depends on the number of generations for which a population remains small, the amount of loss of genetic variation, and the strength of the relationship between population size and population genetic diversity may provide insight into the timing of the fragmentation.

The characteristic longevity of tree species will initially buffer the loss of genetic diversity in fragmented habitats (Hamrick 2004), but trees are eventually as likely to exhibit negative genetic responses to habitat fragmentation as short lived plant species (Vranckx *et al.* 2012). The average estimation of maximum ages for four *Polylepis* species is *c.* 250yr (Roig *et al.*

2001; Argollo *et al.* 2006; Suarez *et al.* 2008; Gareca *et al.* 2010a, see Chapter 6), with a time until first reproduction of *c.* 30yr (Gosling *et al.* 2009). This implies that if *Polylepis* woodlands were naturally occurring in relatively small and isolated fragments, at least since the end of the latest ice age (18,000 BP), then current populations can be expected to be highly genetically differentiated, and to show significant relationships between population size and remaining genetic diversity. If, on the contrary, *Polylepis* woodlands had a more continuous distribution in the past, and fragmentation is of more recent human origin, patterns of genetic structuring can be expected to be less pronounced, and even to disappear when the main fragmentation events would have coincided with the Spanish conquest, *c.* 400yr BP. If fragmentation is of very recent origin, relative to the generation time of the trees, it can also be expected that the genetic signature of habitat fragmentation is mainly apparent in the offspring, and not in the adult generation (Vranckx *et al.* 2012).

The aims of this study were to: (1) present a synthesis of all available studies investigating patterns of within and between population genetic variation in *Polylepis* woodlands throughout the high Andes; and (2) to make inferences regarding the timing of the fragmentation of the Andean *Polylepis* woodlands. Before synthesizing all available population genetic data on *Polylepis* species from the literature, we first present the results of an additional, AFLP based, population genetic study on *P. besseri* Hieron., that we performed in the high Bolivian Andes.

Materials and methods

Study species and study sites

Polylepis Ruiz & Pav. (Rosaceae) species are likely wind pollinated. Self pollination through geitonogamy may occur (Simpson 1979) although decreased germination rates following selfing have been reported for *P. australis*, Bitter (Seltmann *et al.* 2009). Each fruit is typically a one-seeded achene, which is also the dispersal unit (Simpson 1979). Most species have hard fruits with few ridges or knobs which do not seem to be particularly well adapted for any kind of long-distance transport. Results on fruit dispersal and regeneration suggest a low seed dispersal capacity (<8m) for *P. australis* (Torres *et al.* 2008), *P. incana* Kunth and *P. pauta* Hieron. (Cierjacks *et al.* 2007b).

Our own study species, *Polylepis besseri* Hieron., is a tree that grows 8m tall between 3000 and 4100m a.s.l. in the high Andean region of Chuquisaca and Cochabamba, Bolivia (Fjeldså & Kessler 1996). It covers only two percent of the 1000km^2 estimated as suitable habitat for the species in Cochabamba (Fjeldså & Kessler 1996) and it has a vulnerable conservation status (World Conservation Monitoring Centre 1998a, see Chapter 2). Genome size measurements suggest it is an hexaploid species that belongs to the *incana* group of the genus (Schmidt-Lebuhn *et al.* 2010).

The study site included two basins, Sacha Loma and Cuturi (17º 44'S, 65º 34'W, Fig. 7.1), with 14 well delimited monospecific woodlands. The woodlands differ in size, shape and connectivity (Fig. 7.1, Table 7.1). Connectivity was measured as:

$$S_i = A_i^c \sum_{j \neq i} \exp(-\alpha d_{ij}) A_j^b$$

following Moilanen & Nieminen (2002) using the edge to edge distances of the fragments (d_{ij}), and where Ai and Aj are the areas of fragment i and j, respectively, and the parameters b and c represent the scaling of emigration and immigration as a function of fragment area. We used the values of c = 0.3, b = 0.5 as recommended by Moilanen & Nieminen (2002) and α = 5, 0.5 and 0.2. These α-values represent average dispersal distances of 200m, 2 and 5km, respectively. All connectivity values were highly correlated (>97%), therefore α = 0.5 was used for all subsequent analyses.

Sampling scheme and DNA extraction

In each of the 14 woodlands, young leaf material was collected from 20 randomly chosen adults. Additionally, in each of six woodlands (three small: S, R and I, and three large: A, B and C, Fig. 7.1), young leaf material was collected from 20 randomly chosen saplings (<20cm in height). Sampled leaf material was immediately stored in paper bags with silica gel. Before DNA extraction, 5mg of leaf material was homogenized with a mill to fine powder. Total DNA was extracted from this powder using the QuickPick™ Plant DNA kit following the instructions of the manufacturer in a MagRo™ System Unit (Bionobile). DNA quality was evaluated on agarose gels (1% w/v) and its quantity was measured using a spectrophotometer (NanoDrop® ND-1000).

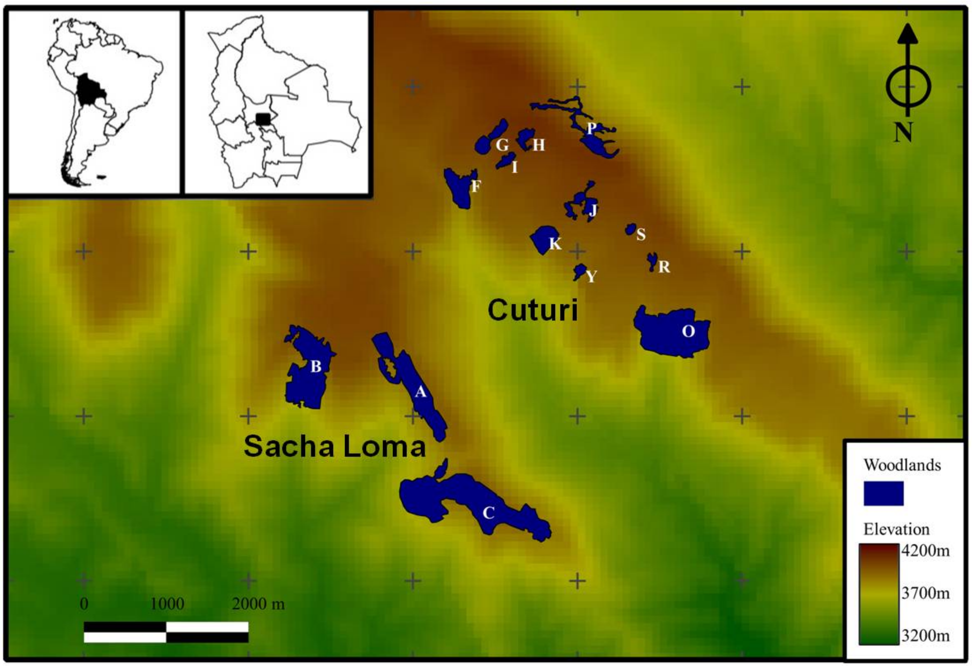

Fig. 7.1 Location of the study area (*insets*). *Blue areas* are *Polylepis besseri* woodlands. *Letters* represent fragment codes and the names of the two studied basins are presented. Colours refer to topography, where *green* represents elevations <3200m, *yellow* 3700m and *brown* 4200m.

AFLP protocol

AFLP analysis was carried out according to Vos *et al*. (1995). Restriction and ligation were performed in a single step. The amplification was performed for every individual with three primer combinations (*Eco*RI-CCA/*Mse*I-GTT, *Eco*RI-CCA/*Mse*I-GGA, and *Eco*RI-CAT/*Mse*I-GCT). Amplified fragments were separated and visualized using denaturing acrylamide gels on a Nen IR2 DNA analyzer (Li-Cor, Lincoln, Nebraska, USA). Sizes of fragments were

Table 7.1 Woodland characteristics and genetic data for the adults and saplings of *Polylepis besseri* in two Bolivian basins.

Basin	Woodland	Area (ha)	Population size	Connec-tivity	Adults				Saplings			
					n	*PPL*	H_e	*MV*	n	*PPL*	H_e	*MV*
Sacha	A	30.4	46,973	55.04	20	77.38	0.278	11.8	17	85.54	0.302	13.03
Loma	B	34.4	66,973	40.61	20	83.33	0.315	13.5	20	55.42	0.174	7.23
	C	65.3	93,891	48.41	20	86.9	0.265	11.88	20	65.06	0.212	8.65
Cuturi	F	10.5	18,302	36.20	20	55.95	0.183	7.2	0	-	-	-
	G	5.5	6075	26.73	20	90.48	0.268	11.14	0	-	-	-
	H	2.8	2267	31.60	20	88.1	0.281	12.17	0	-	-	-
	I	2	2396	25.31	20	90.48	0.299	13.23	16	78.31	0.276	12.18
	J	6.1	5268	38.95	20	89.29	0.315	13.74	0	-	-	-
	K	7.2	8094	43.10	20	71.43	0.240	9.86	0	-	-	-
	O	40.8	69,647	20.55	20	72.62	0.243	10.14	0	-	-	-
	P	13.2	24,953	20.35	19	61.9	0.203	8.01	0	-	-	-
	R	1.4	875	38.36	20	72.62	0.233	9.99	13	83.13	0.281	12.84
	S	1.2	1500	38.29	19	84.52	0.275	11.81	19	81.93	0.310	12.09
	Y	1.7	809	26.05	20	82.14	0.259	11.13	0	-	-	-
Average	-	15.89	24,859	34.97	-	79.08	0.261	11.11	-	74.90	0.259	11.00

n, number of individuals sampled; *PPL*, percentage of polymorphic loci; H_e, expected heterozygosity for $F_{IS} = 0.5$, *MV*, molecular variance.

determined by the IRDye size standard (50–700bp). AFLP profiles were scored for the presence or absence of bands using the SAGAmx software (Li-Cor). Only fragments between 100 and 600 bp were scored, resulting 83 scored loci.

Data analysis

Genetic diversity within *P. besseri* woodlands was measured as the percentage of polymorphic loci at the five percent level (*PPL*), expected heterozygosity (H_e) and as the molecular variance (*MV*) per population. Allele frequencies were estimated using a Bayesian approach (Zhivotovsky 1999) and H_e was estimated following Lynch and Milligan (1994) using AFLPsurv (Vekemans *et al.* 2002). H_e was calculated for different values of F_{IS} (0, 0.3, 0.5, 0.7, 0.9 and 1), since no data regarding the level of inbreeding within populations was available. *MV* was defined as the sum of squares within each population obtained from an AMOVA, divided by the number of individuals sampled per population *minus* 1 (Fischer & Matthies 1998). Spearman rank correlations were calculated between *PPL*, H_e and *MV*, and fragment characteristics (population size and connectivity).

Genetic differentiation among *P. besseri* woodlands, measured as Φ_{ST}, was obtained through AMOVA. Significance was tested using 999 random permutations. The two first principal coordinates of a Principal Component Analysis (PCA) on the matrix of pairwise genetic differentiation (pairwise Φ_{ST}), were derived. To test for isolation by distance, a Mantel test was performed between the matrices of pairwise genetic differentiation and the edge-to-edge geographic distances between fragments, applying 999 permutations to test the significance of the correlation. Mantel tests were performed for all fragments, and for the fragments in the Cuturi basin (n= 11) only to test for isolation by distance at a local scale. *PPL*, AMOVA analysis, Φ_{ST}, and Φ_{SC} (see below) were calculated in GenAlEx 6.4 (Peakall & Smouse 2006).

To study the evolution of genetic diversity across generations (adults and offspring), the significance of overall differences in *PPL*, H_e and *MV* between adults and saplings was evaluated using a paired Wilcoxon test for the six fragments with sapling information. Then, for each fragment, we calculated the difference in *PPL*, H_e, and *MV* between adults and saplings. Mann-Whitney tests for independent samples were performed on these differences to compare large (n= 3) *vs*. small (n= 3) fragments. In order to compare the genetic differentiation across generations, the Φ_{ST} values were obtained for adults and saplings in the same six fragments.

Literature search

We performed a search in the ISI Web of Knowledge and Google Scholar with the terms "*Polylepis*" AND "Andes" AND "population genetic*" in January 2012. The minimum and maximum geographic distances between woodlands per study were estimated from the maps available in the publications. In order to be able to compare different studies, the ratios (*PPL* offspring)/(*PPL* adults) and (H_e offspring)/(H_e adults) were calculated to detect intergenerational changes in genetic diversity. Φ_{ST}, a measure of the correlation of a pair of individuals drawn at random from within populations, relative to the correlation of a pair of individuals drawn at random from the whole dataset (Lowe *et al.* 2004), was selected as a measure of genetic differentiation at the regional scale. In order to control for the large differences in the distances between regions a second measure of genetic differentiation, Φ_{SC} was used as the genetic differentiation measure. Φ_{SC} measures the correlation of a pair of individuals drawn at random within populations relative to the correlation of a pair of individuals drawn at random within region, and it was defined as $\Phi_{SC} = \sigma_b^2 / (\sigma_b^2 + \sigma_c^2)$ where σ_b^2 is the variance among populations and σ_c^2 is the variance among individuals within populations (Lowe *et al.* 2004).

Results

Population genetics of *Polylepis besseri*

On average, *Polylepis besseri* adults had 79.1% of polymorphic loci (± 2.9, SE). H_e values for different F_{IS} were highly correlated (>92%) and we continued the analysis with H_e values for $F_{IS}= 0.5$. Average H_e over all populations was 0.26 (± 0.01, SE). Overall molecular variance was 11.1 (± 0.5 SE) (Table 7.1). There was no correlation between *PPL*, H_e and *MV*, and fragment characteristics (population size and connectivity) ($P> 0.1$).

The AMOVA results showed that most of the genetic variation was present within populations (82.7%), followed by the among regions component (9.5%), and among populations within a region component (7.8%). The Φ_{ST} for adults for all fragments (n= 14) was 0.173 ($P= 0.001$). Pairwise Φ_{ST} values ranged from 0.012 to 0.332 (Table 7.2). The PCA diagram for adults showed that woodlands from Sacha Loma clustered at the right hand part of the diagram, while most Cuturi fragments were on the left or middle (Fig. 7.2). Mantel tests demonstrated significant isolation by distance at a regional scale when all woodlands were included ($r_M= 0.299$, $P= 0.02$, Fig. 7.3a), but not when only woodlands from the Cuturi basin were used in the analysis ($r_M= -0.095$, $P= 0.26$, Fig. 7.3b).

The comparison of genetic diversity across generations for the six fragments resulted in non-significant differences for the *PPL* (average for adults 82.5% and for saplings 75.1%, $P= 0.25$), H_e (average for adults= 0.278 and for saplings 0.259, $P= 0.75$) and *MV* (average for adults 12.04 and for saplings 11.00, $P= 0.60$). Large and small fragments showed no significant differences with respect to genetic diversity measures (Mann-Whitney tests $P> 0.10$). Genetic differentiation in the same six fragments was $\Phi_{ST}= 0.13$ for adults, and $\Phi_{ST}= 0.12$ for saplings.

Population genetics of *Polylepis*

Including this study, we retrieved seven papers dealing with genetic diversity of *Polylepis* woodlands (Table 7.3). They included *Polylepis* species from Argentina (*P. australis*), Bolivia (*P. besseri*), Peru (*P. multijuga* Pilg.) and Ecuador (*P. incana* and *P. pauta*), using allozymes, RAPD, ISSR, AFLP and cpDNA as genetic markers (Table 7.3). Four studies reported genetic diversity of both adults and offspring (Table 7.3). The distance between woodlands ranged from 0.3 to 1154km (Table 7.3).

Table 7.2 Genetic differentiation, measured as Φ_{ST}, and its statistical significance between *Polylepis besseri* woodlands in Bolivia.

	A	B	C	J	H	G	I	F	O	R	S	P	K	Y
A		0.093	*	*	*	*	*	*	*	*	*	*	*	*
B	0.031		*	0.061	*	*	*	*	*	*	*	*	*	*
C	0.044	0.051		*	*	*	*	*	*	*	*	*	*	*
J	0.063	0.042	0.061		0.178	*	*	*	*	*	*	*	*	*
H	0.097	0.066	0.048	0.014		0.143	0.070	*	*	*	*	*	*	*
G	0.168	0.136	0.097	0.086	0.018		*	*	*	*	*	*	*	*
I	0.139	0.1	0.064	0.065	0.037	0.039		*	*	*	*	*	*	*
F	0.332	0.271	0.235	0.244	0.163	0.078	0.089		*	*	*	*	*	*
O	0.249	0.215	0.159	0.183	0.122	0.044	0.045	0.036		*	*	*	*	*
R	0.186	0.173	0.102	0.124	0.093	0.076	0.062	0.145	0.059		0.157	*	*	*
S	0.183	0.166	0.082	0.13	0.086	0.053	0.049	0.14	0.028	0.012		*	*	*
P	0.301	0.285	0.21	0.235	0.163	0.095	0.128	0.136	0.05	0.097	0.036		*	*
K	0.233	0.177	0.172	0.161	0.125	0.083	0.115	0.168	0.114	0.116	0.119	0.139		0.131
Y	0.175	0.137	0.104	0.116	0.089	0.054	0.07	0.143	0.086	0.086	0.081	0.122	0.016	

P-values are presented in the upper right triangle of the matrix. * indicates significant differences ($P< 0.05$).

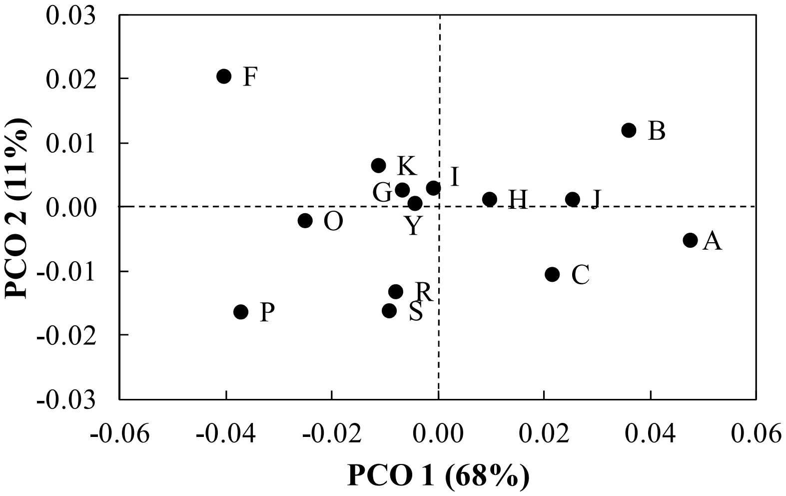

Fig. 7.2 Principal coordinates of the pairwise Φ_{ST} genetic distances between fragments. *Values in parenthesis* represent the percentage of explained variability by each principal coordinate. *Letters* represent fragment codes.

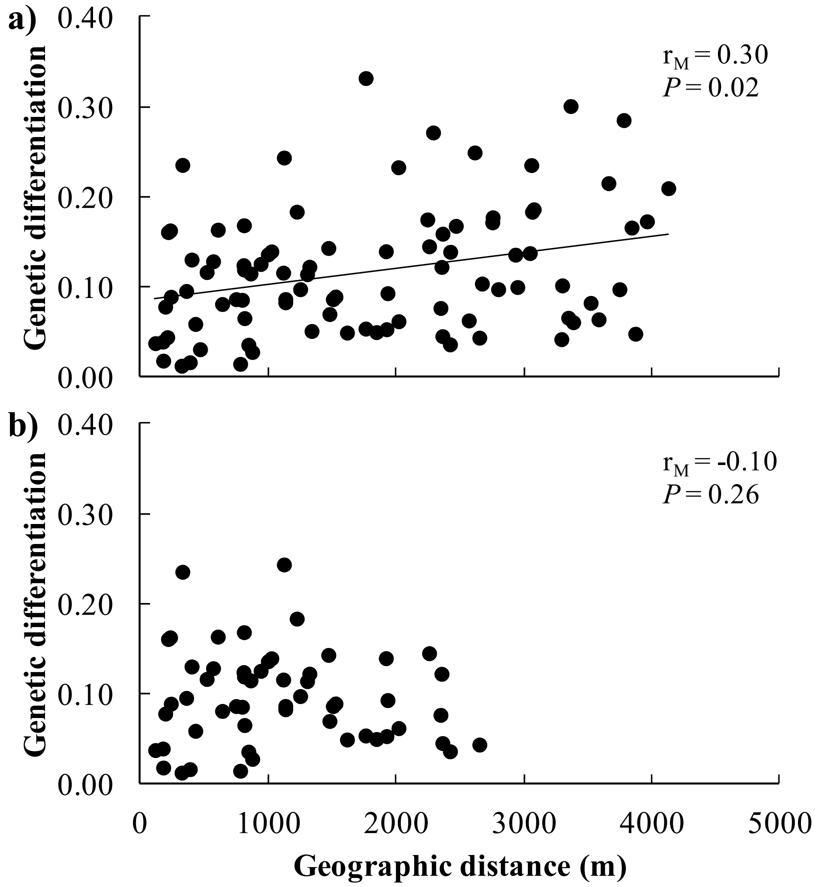

Fig. 7.3 Relationship between genetic differentiation and geographic distance (a) for all woodlands, and (b) for woodlands from Cuturi basin. Mantel correlation values and their probabilities are shown. The *trend line* does not indicate causality.

TABLE 7.3 Overview of studies found regarding *Polylepis* population genetics in the high Andes.

Species	Number of woodlands (regions)	Woodland area range (ha) (mean)	Min-max distance (km)	Sample size adults (offspring)	Marker type	*PPL* adults (off-spring)	H_e adults (offspring)	r_S	Φ_{ST} for adults (offspring)	Φ_{SC} for adults (offspring)	Ratio *PPL*	Ratio H_e	Reference
P. besseri	14 (2)	1.2-65.3 (15.6)	0.3-4.8	278 (105)	AFLP	79.1	0.261	-0.27	0.17	0.09			This study
	6 (2)		0.3-4.8	119 (105)	AFLP	82.5 (74.9)	0.278 (0.259)		0.13 (0.12)	0.04 (0.05)	0.91	0.93	
P. multijuga	9 (4)	<1- ~200 (-)	0.5-79	371 (0)	AFLP +cpDNA	54.9	0.130	-	0.13	0.13	-	-	Quinteros-Casaverde *et al.* (2012)
P. incana	10 (3)	0.8-71 (21.8)	0.9-36.4	118 (118)	AFLP	44.8 (38.6)	0.145 (0.115)	-0.02	0.31 (0.30)	0.16 (0.18)	0.86	0.79	Hensen *et al.* (2012)
P. australis	18 (3)	80-700 (216)	5-1154	208 (0)	AFLP	54.97	0.181	-0.04	0.16	0.08[a]	-	-	Hensen *et al.* (2011)
P. pauta	12 (3)	1-55 (16.6)	0.3-14.3	287 (288)	Allozy-mes	73.7 (73.7)[b]	0.294 (0.291)	0.17[b]	0.06 (0.14)	0.05 (0.12)[a]	-	0.99	Aragundi *et al.* (2011)
P. australis	5 (5)	-	1.2-31.2	90 (0)	ISSR	90.76	0.376[c]	-	0.02	-	-	-	Julio *et al.* (2011)
P. australis	2 (2)	-	34.0-34	20 (20)	RAPD +ISSR	88.5 (88.5)	0.258 (0.258)	-	0.02 (0.08)	-	-	-	Julio *et al.* (2008)
Average	-		6.0-193	-	-	69.5 (68.9)	0.240 (0.231)	-	0.12 (0.16)	0.10 (0.12)	-	-	-

Min-max distance, minimum and maximum distance between woodlands; *PPL*, Percentage of polymorphic loci; H_e, expected heterozygosity, r_S Spearman correlation between *PPL* and fragment area for adult individuals; Ratio *PPL*, (*PPL* offspring)/(*PPL* adults); Ratio H_e, (H_e offspring)/(H_e adults).

* Significant at the 0.05 level ($P < 0.05$).

[a] Estimated as the average of the among populations variation for the studied regions.

[b] For adults and offspring together.

[c] Estimated as the average of the marker diversity index (M) obtained per population.

Polylepis incana and *P. australis* populations had the lowest and highest *PPL* values, respectively (44.8% and 90.8%, Table 7.3). Four out of the seven studies found *PPL* values larger than 70 percent, while three reported values between 40-60% (Table 7.3). H_e values ranged from 0.145 (*P. incana*) to 0.376 (*P. australis*) with an average H_e of 0.240 across species (Table 7.3). The offspring of *P. besseri* (*PPL* adults= 82.5%, offspring= 74.9%) and *P. incana* (*PPL* adults= 44.8%, offspring= 38.6%), both showed a decrease in genetic diversity compared to that of the adults. *P. pauta* (Φ_{SC} adults= 0.05, Φ_{SC} offspring= 0.12) and *P. incana* (Φ_{SC} adults= 0.16, Φ_{SC} offspring= 0.18) offspring showed an increase in differentiation at the local scale compared to the adult generation. *P. pauta* (Φ_{ST} adults= 0.06, Φ_{ST} offspring= 0.14) and *P. australis* (Φ_{ST} adults= 0.02, Φ_{ST} offspring= 0.08) offspring had two and four times more genetic differentiation at the regional scale compared to the adults (Table 7.3).

Discussion

Genetic diversity within *Polylepis* woodlands

If *Polylepis* woodlands were naturally highly fragmented since the beginning of the Holocene, then population genetic drift in the small and isolated woodlands could be expected to have caused severe loss of genetic diversity. Six out of the seven studies, however, found *PPL* values higher than 55 percent. These values are well within the range of the average values reported for plant species with a mixed-mating system and with seeds dispersed by gravity (42%, n= 178) or wind (53%, n= 186) (Hamrick & Godt 1996). The H_e values we retrieved are generally also well above the averages reported for long lived perennial species (0.25, n= 37), species with outcrossing (0.27, n= 38) or mixed mating system (0.18, n= 8), species with seeds dispersed by gravity (0.19, n= 24) or wind/water dispersal (0.27, n= 7), and late successional species (0.30, n= 16) (Nybom 2004). Only one out of 7 species in our literature review (*P. incana*) had an H_e value of 0.14, much lower than these previously reported values. This at least suggests that genetic diversity of fragmented *Polylepis* woodlands generally shows no signature of severe erosion.

Furthermore, the four correlation coefficients between population genetic diversity and fragment area were not significant (Table 7.3). Under the assumption that woodland area is a good proxy of the size of the *Polylepis* populations, and that genetic diversity is lost at a rate that is inversely related to population size, these findings do not support loss of genetic variation in the smallest populations. It has been suggested that longevity buffers the effects of genetic drift in small populations (Hamrick 2004). However, in a recent meta analysis, Vranckx *et al*. (2012) have shown that significantly positive relationships between population size and genetic diversity are very common in woody plant species subjected to habitat fragmentation, even after few generations. Alternatively, theory also predicts that polyploid plant species may be less subjected to drift than diploid species (Moody *et al.* 1993), but in a recent meta analysis of the genetic diversity in 101 plant species occurring in fragmented habitats, Aguilar *et al*. (2008) could not find empirical evidence for this prediction.

Genetic differentiation among *Polylepis* woodlands

The genetic differentiation between *Polylepis* populations at the local scale (Φ_{SC}) was consistently lower than average values of population genetic differentiation that have been reported for long lived perennial plants (0.25, n= 60), endemic species (0.26, n= 22), species with outcrossing (0.27, n= 73) or mixed (0.40, n= 18) breeding systems, wind/water (0.25, n= 22) or gravity (0.45, n= 46) seed dispersal and of late successional status (0.23, n= 29) (Nybom 2004). With the exception of *P. incana* (Φ_{ST}= 0.31), similar values were observed at the regional scale for most *Polylepis* species (Φ_{ST}, Table 7.3). Because population genetic differentiation is a direct consequence of genetic drift in small populations, as alleles are randomly lost across populations, these results are in accordance with minor losses of within population genetic diversity, and with the lack of a significant relationship between woodland fragment area and genetic diversity. Alternatively, high gene flow through seeds or pollen between populations can replenish alleles that have been lost through drift, in turn reducing genetic differentiation between populations (Honnay *et al.* 2007). Vranckx *et al*. (2012), however, have recently shown that tree species that are wind pollinated, which suggest a high capacity to exchange pollen between populations, are as sensitive to loss of genetic variation through habitat fragmentation as insect pollinated tree species. It is likely that due to the leptokurtic dispersal of wind-borne pollen, the efficiency of wind pollination sharply decreases as distances from a pollen source increase (Honig *et al.* 1992). Once a threshold value of spatial isolation has been reached, less pollen will be transferred successfully between forest fragmented populations. All together, the patterns of within and between genetic diversity in fragmented *Polylepis* suggest a rather recent fragmentation history. There seems to be one major exception, *P. incana*. Adult individuals of *P. incana* showed the lowest values for *PPL* (45%) and H_e (0.145), and the highest genetic differentiation between woodlands (Φ_{ST}= 0.31).

Genetic differences between offspring and adults

When comparing genetic diversity of the offspring generations with the adult generations, two studies provided data for estimating the ratio (*PPL* offspring)/(*PPL* adults), and three allowed us to calculate the ratio (H_e offspring)/(H_e adults). Results suggest a decrease of genetic diversity from adults to offspring for *P. incana* (Hensen *et al.* 2012). Additionally, Hensen *et al.* (2012) found a significant relationship between genetic diversity and population size in the offspring of *P. incana*. Negative genetic consequences of habitat fragmentation were also reported for *P. pauta* and *P. australis* offspring. *P. pauta* showed an increase in Φ_{ST} of two times when comparing offspring and adult genetic differentiation (Aragundi *et al.* 2011), and *P. australis* even showed an increase of four times (Julio *et al*. 2008). These results indicate that *Polylepis* woodlands are indeed suffering from genetic erosion but that the signature of genetic drift is mainly visible in the recently produced offspring, and not in the adult generation. This suggests that the populations are not in a genetic equilibrium yet and that the main habitat fragmentation event is of recent origin, relative to the generation time of *Polylepis* species. As found by Vranckx *et al.* (2012), long-lived tree species are as likely to have negative genetic responses to habitat fragmentation as short-lived species. Genetically impoverished offspring has also been reported for *Fagus sylvatica* L. (Jump & Peñuelas 2006), *Araucaria nemorosa* de Laub. (Kettle *et al*. 2007), and *Quercus ilex* L. (Ortego *et al.* 2010).

Conclusion

We found no genetic evidence of long term fragmentation effects in the adults of the studied *Polylepis* species (except for *P. incana*). If the current fragmentation status of the *Polylepis* woodlands has lasted for many thousands of years, more genetic differentiation and less within population genetic diversity would have been observed, as well as a positive relationship between genetic diversity and population size. Even wind pollinated species have indeed been reported to be subject to loss of genetic variation in small populations (see Jump & Peñuelas 2006; Vranckx *et al.* 2012). Whereas most studies found no significant relationship between fragment size and genetic diversity in the adults, three studies reported decreased genetic diversity or an increase in genetic differentiation in the offspring. All together, the available population genetic data suggests that fragmentation effects are only starting to become apparent, and that the area and spatial isolation threshold where fragmentation effects are starting to become visible in the genetic makeup of the species has been reached relatively recently. The losses of *Polylepis* woodlands have been suggested to have occurred 5000-3000yr BP and following the beginning of the Spanish conquest (AD 1533) (Kessler & Driesch 1993). Based on our results, we support the second hypothesis, *i.e.* we suggest that this threshold has been passed *c.* 400yr BP, coinciding with the colonization of South America by the Europeans. Our results do not suggest that fragmentation did not occur at all before this period, but that the area and connectivity of *Polylepis* fragments has always been sufficient to counteract genetic erosion. Although our results support the anthropogenic fragmentation hypothesis for *Polylepis* woodlands in the high Andes, they also suggest that the main fragmentation event occurred much later than previously suggested (Kessler 1995; Fjeldså & Kessler 1996). The much better seed germination in the grassland as compared to in *Polylepis* woodlands (Chapter 4) seems to be a strong indication that the potential area for *Polylepis* woodlands is much higher and that the fragmentation is of recent origin.

The presence of enough connectivity between *Polylepis* woodlands sufficient to counteract genetic erosion through time found in this Chapter seems to contradict the possibility for local adaptation at high elevations found for *P. besseri* (Chapter 4). However, it has been reported that local adaptation occurred along an altitudinal gradient for plant survivorship, diameter, height, leaf dry matter content, and specific leaf area, even in the presence of gene flow along three altitudinal gradients (from 1500 to 2500m) in the alpine plant species *Festuca eskia* Ramond ex DC. (Poaceae) (Gonzalo-Turpin & Hazard 2009). Similar results were reported for the sessile oak *Quercus petraea* (Matt.) Liebl. (Fagaceae). In this case, local adaptation along two altitudinal transects from 131 to 1630m for leaf unfolding and germination timing was reported (Alberto *et al.* 2011), despite the fact that pollen flow (which was mainly from lower to higher elevations) contributed to the maintenance genetic diversity along the studied transects (Alberto *et al.* 2010).

In order to avoid further genetic impoverishment of *Polylepis* woodlands, manual introduction of pollen or seed from other fragments can be thought of as an efficient conservation strategy. A prudent approach would be to do these introductions on an experimental basis, monitoring survival and reproductive success of natural and manual crosses (Frankham *et al.* 2002). A consequence of our findings is that larger areas of the high Andes may support *Polylepis* woodlands than the area reflected by the current distribution of the woodlands. This presents an immense challenge for ecosystem conservation and restoration, not only because of the required restoration practices themselves, but also because of the need of adequate governmental policies (Kessler 1998). These findings also offer opportunities regarding carbon sequestration projects in the high Andes, not because of the fast growth rate of *Polylepis* trees (*e.g.* Gareca *et al.* 2010a, see Chapter 6), but because of the area they may occupy; in Bolivia *Polylepis* occupies only 11% of the 51,000km^2 of their potential distribution area (Kessler 1995). It is worth to mention that most *Páramos* in the Northern Andes store large amounts of carbon in their soils (*i.e.* they are considered a global carbon sink) because of a continuous vegetation cover (mainly due to grassland), low air temperature, the low atmospheric preasure, frequent water logging of the soils, and by the formation of resistant complexes with Al and Fe (Buytaert *et al.* 2006b; 2011). The organic matter accumulation generates an open and porous soil texture, which has a large water storage capacity, and that is highly sensible to livestock trampling or mechanical cultivation (Buytaert *et al.* 2006b). However, if trees were grown in the *Páramos*, then a potential loss of below-ground carbon storage may be compensated by increased above-ground storage (Buytaert *et al.* 2011). It has been shown that total above ground biomass in an old growth forest of *Polylepis incana* in the *Páramo* (366Mg/ha) can be compared with those values for species from the lowland tropical forests (Fehse *et al.* 2002). Studies from the *Puna* region revealed that total soil carbon storages on average were not statistically different among grassland, shrubland and tropical montane cloud forest sites across the treeline in the Peruvian Andes (Zimmerman *et al.* 2010). However, the ratio belowground: aboveground carbon was higher on the grassland compared to the transition zone (shrubland) and the upper tropical montane cloud forest sites (forest, Gibbon *et al.* 2010). Thus, carbon stocks in the puna soils are more important than in the forests, and their fate under an afforestation (or reforestation) scenario needs to be studied.

CHAPTER 8

General discussion and conclusions

Summary of the results

Polylepis woodland biodiversity in Bolivia

The high Andes of Bolivia holds 13 species and three subspecies of the genus *Polylepis* (Chapter 2). Based on 31 publications, we found 780 species inhabiting the *Polylepis* woodlands: 425 plants, 266 birds, 46 mammals, 35 butterflies and 8 reptiles. Ten of the 13 Bolivian *Polylepis* species, as well as 7 other plant species, 14 bird species and 4 mammal species were categorized as threatened or near threatened according to IUCN criteria (Chapter 2).

Most of the plants found in the *Polylepis* woodlands were herbs (40%), followed by shrubs, grasses, ferns, and others. Almost half of the birds were insectivorous, followed by frugi-granivorous and nectarivorous species. Insectivorous mammals were also the most abundant followed by herbivorous and nectarivorous. The reptiles were insectivorous and carnivorous. The only study dealing with insects was done on Lepidoptera and most of them were nectarivorous, although their larvae are herbivorous (Chapter 2).

We found a positive association between plant species richness *vs.* precipitation and length of the growth season, and a negative relation between plant species richness and elevation (Chapter 2). A higher length of the growth season and a higher amount of water (precipitation) are related with a higher system productivity, which might lead to increased plant species richness (Begon *et al.* 1996). For birds, we found a positive correlation between total species richness and precipitation, as well as length of the growth season (Chapter 2). The higher plant species richness may offer more resources and niches for birds, increasing their species richness. The highest bird endemism in *Polylepis* woodland remnants occurred at intermediate elevations, temperatures and precipitation. Mammal species richness decreased with increasing maximum temperature (Chapter 2).

Regeneration ecology of *P. besseri* under both lab and field conditions and inferences regarding climate warming effects and exotic species

Hydrothermal time models for *P. besseri* could be assembled fairly straightforward due to the lack of a seed dormancy mechanism (Chapter 3). We estimated the hydrothermal time model parameters for the seed germination of *P. besseri* and found that seed germination is not limited by the maximum temperature in the field at present, but by the minimum temperature (Chapter 3). The base temperature for *P. besseri* was estimated as 3.0°C and the optimum temperature 21.7°C (Chapter 3). The water potential threshold preventing radical emergence for 50% of the germinated seeds was -0.74MPa ($\psi_{b(50)}$) and a standard deviation of 0.41MPa ($\sigma_{\psi b}$). Fluctuating temperatures did not stimulate germination; on the contrary, they resulted in less germination in the 20/2°C treatment. The HTT model developed for the germination of *P. besseri* at constant temperatures also performed well in predicting germination at the fluctuating 20/10°C treatment; however the model performed poorly to predict germination in the fluctuating 20/2°C treatment, which included temperatures below the base temperature (Chapter 3).

Seed germination success in the field depended on two factors: the source elevation of the seeds and the sowing elevation (Chapter 4). Total germination of seeds from 3600m increased with decreasing elevation (*i.e.*

increasing temperature), whereas seeds from high elevations (3800m) showed their maximum total germination at low (3400m) and high elevations (3800m). Moreover, seeds from high elevations showed a home-site advantage, suggesting a pattern of local adaptation (Chapter 4). The germination rate increased with decreasing elevation. Facilitation could not be detected at any of the three altitudes studied, since a higher cumulative germination was observed in the grassland compared to the germination under a nurse tree or shrub (Chapter 4). Finally, seeds from 3800m and 3600m showed similar HTT model parameters (Chapter 4). A comparison of laboratory and field experiments regarding germination is not possible due to the lack of microclimatic data. Specifically, measurements of soil water potential and soil temperatures during germination period would be needed to allow this comparisons and possible predictions on seed germination in the field.

Regarding *P. subtusalbida*'s regeneration, we found that mixed fragments with both native and exotic trees, and native pure fragments did not present differences in seedling density (Chapter 5). Differences in density were related to adult native tree densities. The size structure only varied between locations and not between fragment-types. A greater proportion of *P. subtusalbida*'s seedlings exhibited lateral growth or lateral growth with adventitious roots in mixed fragments than in pure fragments. Smaller individuals grew more, in both pure and mixed fragments. Greater survival of seedlings was observed in the larger individuals, in the ones with vertical growth, and in those that exhibited lateral growth with adventitious roots (Chapter 5).

Regional climate *vs*. radial growth relationships for *P. besseri*

Wood samples of *P. besseri* exhibited semicircular porosity and visible tree-ring boundaries (Chapter 6). The sample trees were young (mean age 40yr, maximum age 63yr) and growing slowly (<1mm/yr). Trees at Chutu Senega were older and growing more slowly than those at Uypa. The strong linearity of cumulated radial increments suggests that *P. besseri* may maintain consistent growth rates beyond 60yr. Our results indicate that radial growth is limited by the accumulation of reserves the year before ring formation, and that a warm period before the growing season (humid period) can increase *P. besseri*'s growth at Uypa, whereas an increase of rain may limit radial growth in both studied fragments (Chapter 6). It seems that local factors are more important than regional factors for radial growth in these high Andean woodlands. Although a significant correlation between El Niño-Southern Oscillation and growth rate was found, this was not the same for both studied fragments.

Population genetics of fragmented *Polylepis* woodlands

Including our study, we found seven publications on the population genetics of five *Polylepis* species (Chapter 7). Adult estimates of population genetic diversity were high compared to other similar plant species. We did not find a general relationship between population size and genetic diversity, and the genetic differentiation was low compared to these reported for similar plant species. Analysis of the offspring, however, showed loss of genetic diversity and increasing differentiation compared to the adult generation. We could not find evidence in support of a history of long term fragmentation. Genetic erosion of the offspring compared to the adult generation, on the contrary, suggests that populations are not in genetic equilibrium yet, and that the main habitat fragmentation event is of relatively recent origin (*c.* 400yr).

Our research focussed on the effects of three major threats to the economically and ecologically important high-Andean genus *Polylepis*. We studied how global warming and exotic tree species affect seed germination, growth and survival of *P. besseri* and *P. subtusalbida* in Bolivia; as well as how habitat fragmentation affects the genetic structure of *Polylepis.* We can summarize the major conservation threats as follows.

Consequences for conservation and restoration

Regeneration ecology of *P. besseri*

The HTT models allowed us to make a prediction about the germination of *P. besseri* under global warming. An increase in temperature of 0.3°C of the current minimum field temperature (-1.1 to 2.7°C; *c.f.* Grieser *et al.* 2006) may already release *P. besseri* germination from the limit imposed by its base temperature. As temperatures rise, germination will be the result of the balance between increased temperatures and reduced water potential. Finally, when maximum temperature surpasses the optimum temperature for *P. besseri* (*c.* 21.7°C), germination will start to decrease (Chapter 3). The lack of a seed dormancy mechanism for germination suggests that *P. besseri* seeds can be sown directly in the field (Chapter 3). Since *P. besseri* seeds require mainly water and warm temperatures to germinate, restoration of woodlands might be done when more water is available, *i.e.* the rainy season (as in Chapter 4). For restoration of the woodlands, the relatively heavier seeds can be used (Seltmann *et al.* 2007a). In order to make the seeds germinate, greenhouses or germination chambers can be used without the need of alternating temperatures. In this case, the temperatures need to be around the optimum temperature for *P. besseri*, and germination will be complete in 4-6wk (Chapter 3).

Although seeds from 3600m and 3800m showed similar HTT model parameters, the differences in germination success in the field according to the source elevation of the seeds (3600m *vs.* 3800m), suggest that seed origin must be taken into account for restoration practices (Chapter 4). In the face of global warming, seeds from high elevations (3800m) might become maladapted to warm conditions, given their home-site advantage for cold environments, and if this local adaptation pattern is supported through different years, then the translocation of individuals from low to high elevations has to be considered. On the contrary, cumulative germination of seeds from 3600m as well as the germination rate would increase as temperatures rise (Chapter 4).

The absence of changes in density, growth form structure, growth rate and survival of *P. subusalbida*'s seedlings at different locations and fragment-types (with and without exotic species), are evidence that these seedlings are able to coexist with exotic trees (Chapter 5). Likely, this coexistence occurs under low plant densities or low canopy cover of the exotic trees. However, exotic species increased the frequency of lateral growth forms in *P. subtusalbida*, which in turn had the lowest survival (Chapter 5). Our results suggest that any measure for sustainable management of these forests should be contingent to specific locations. The maintenance of the exotic tree species *Pinus radiata* and *Eucalyptus globulus* at Parque Nacional Tunari has to be considered, and general practices such as pruning and cutting of the exotic trees close to young and adult *P. subtusalbida*s ought to be considered.

Regional climate *vs*. radial growth relationships for *P. besseri*

An increase of temperature before the growing season (humid period) in Sacha Loma and Cuturi would increase *P. besseri*'s radial growth at Uypa fragment, but not at Chutu Senega because trees from both fragments respond in different ways to regional climate (Chapter 6). The low annual growth rate of *P. besseri* (<1mm/yr) should be considered in the development of long term management plans and also for environmental education of the local communities (Chapter 6). The indications that local factors (*e.g.* local disturbance, nutrient availability, or microclimate) are more important than regional factors (*e.g.* climate) for these trees in the studied fragments (Chapter 6) make it difficult to recommend precise locations for reforestation using regional approaches, but this might be an advantage for conservation efforts because local factors are easier to modify than regional factors.

Population genetics of fragmented *Polylepis* woodlands

Our literature survey on the effects of habitat fragmentation to the genetic diversity and structure of *Polylepis* suggests that offspring lost genetic diversity and increased its differentiation compared to the adult generation.

This indicates that fragmentation effects on population genetics of *Polylepis* woodlands are only starting to become apparent (Chapter 7). As a consequence, our results support the anthropogenic fragmentation hypothesis for *Polylepis* woodlands, and suggest the main fragmentation event occurred much later than previously suggested (Kessler 1995; Fjeldså & Kessler 1996). In order to avoid further genetic impoverishment, manual introduction of pollen or seed from other fragments can be thought of as an efficient conservation strategy. A consequence of our findings is that larger areas of the high Andes may support *Polylepis* woodlands than the area reflected by the current distribution. To understand the history of *Polylepis* woodland fragmentation is important for reforestation programs. If *Polylepis* woodland fragmentation would be natural, then reforestation with *Polylepis* species in the high Andes would not be recommended. On the contrary, if *Polylepis* woodland fragmentation would be of recent anthropogenic origin, then this presents an immense challenge for ecosystem conservation and restoration; and offer opportunities regarding carbon sequestration projects in the high Andes because of the area *Polylepis* woodlands may occupy. It is known that the soil carbon stocks in the *Páramo* are considerable (Buytaert *et al.* 2006b; 2011) and in the *Puna* they are more important than in the forest (Gibbon *et al.* 2010). If trees were grown in the *Páramo*, then a potential loss of below-ground carbon storage may be compensated by increased above-ground storage (Buytaert *et al.* 2011).

Most of our research on the ecology of *Polylepis* was conducted in the Peruvian Puna biogeographic province in Bolivia, thus the results can be applied to this region. Because of the differences among biogeographic provinces (Navarro & Maldonado 2002), it is not reccomendable to extrapolate our results to other provinces such as the Peruvian-Bolivian Yungas. The results on the *Polylepis* population genetics are for different countries, thus they are more generalizable than the ecological results. In order to generalize our results for other high elevation tropical ecosystems it would be needed a careful comparison such as that carried by Wesche *et al.* (2008).

Unsolved problems and further research

Further research is needed to model the proposed delay to re-start germination below the base temperature in order to predict how germination will be at 20/2°C for *Polylepis besseri* (Chapter 3). It is also needed to confirm the pattern of local adaptation to high elevations in different years (Chapter 4). The higher germination in the grassland *vs.* under a nurse tree or shrub (Chapter 4) suggests that *Polylepis* might colonize the surrounding grassland, and the identification of the limiting factors for this establishment is very important to provide guidelines for conservation and restoration of the woodlands (Chapter 4). It is important to determine the most important bottlenecks for the establishment of *Polylepis*. Demographic studies would be used to get this information. The conditions determining the negative effects of the exotic tree species on *Polylepis* growth form and survival need to be disentangled (Chapter 5), as well as which kind of competition is more important in the relationships between *Polylepis* and the exotic trees (inter or intra-specific competition; competition for resources or interference). The applications of dendrochronology to understand *Polylepis* autoecology is an empty niche that can benefit from further research. With the use of experimental manipulations, observational studies and non-destructive sampling methods (such as micro-cores), the effects of competition of *P. besseri* with surrounding vegetation, as well as how *P. besseri* trees allocate their resources would be studied.

At a national scale, more research is needed for the woodlands dominated by *P. crista-galli*, *P. hieronymi*, *P. incarum*, *P. pacensis*, *P. pauta*, *P. pepei, P. sericea*, or *P. triacontandra*, since no field surveys were reported for these species. Likely, if these field surveys are done, the number of plants reported for *Polylepis* woodlands will increase because the species accumulation curve did not level off yet for plants (Chapter 2). The lack of research on reptiles, insects and Fungi inhabiting *Polylepis* woodlands denotes the need for more research. The importance of insects in these ecosystems should not be overlooked since most of the birds, mammals and reptiles were insectivorous. Although field surveys remain required to acquire complete accounts of species diversity in *Polylepis* woodlands, much more efforts are necessary to get insight into the processes threatening their exceptional biodiversity.

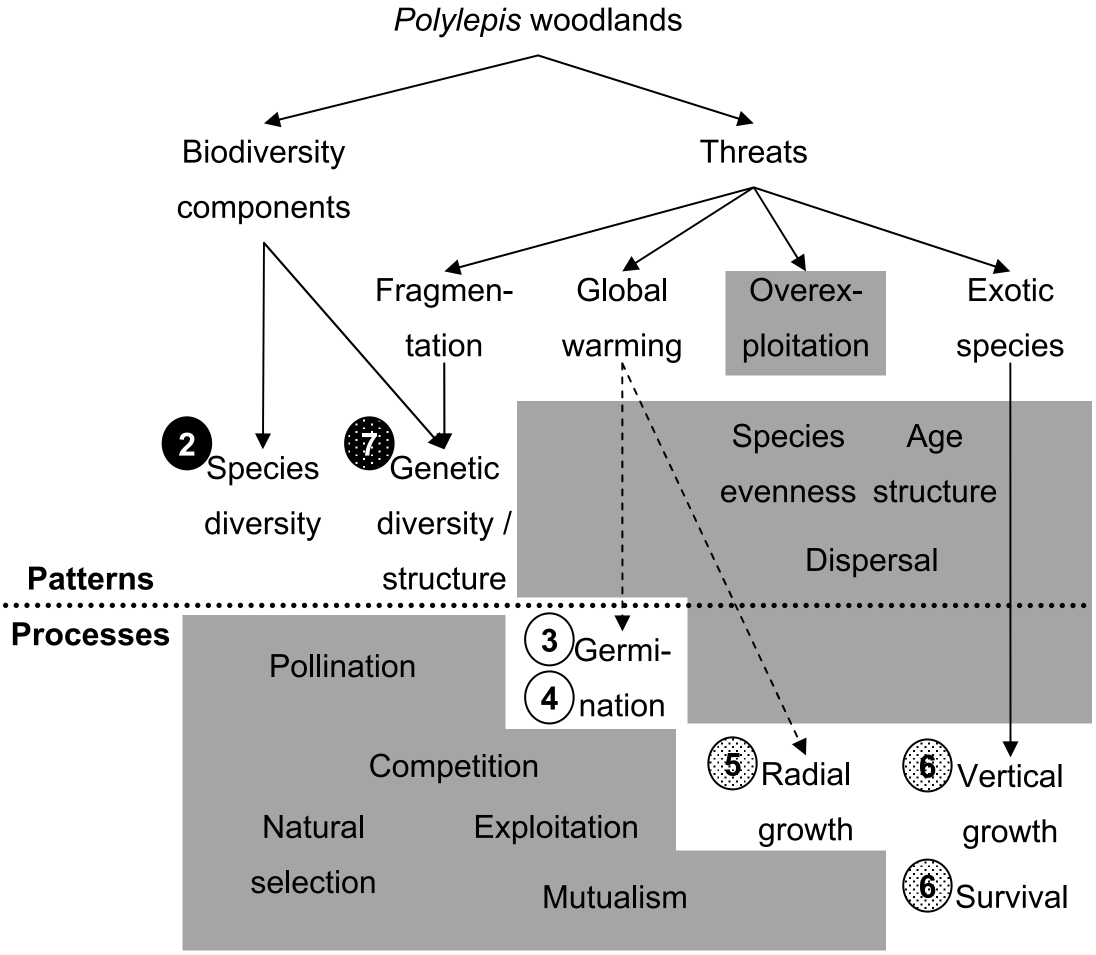

Fig. 8.1 Schematic representation of the *Polylepis* woodlands conservation problem addressed in this study. *Numbers in circles* represent chapter numbers. *Black circles* represent literature reviews, *white circles* represent lab and field experiments, and *dotted circles* are observational studies. *Segmented lines* indicate indirect evidence. Patterns and processes in *gray areas* represent potential research not studied in this work (see text).

Finally, regarding the schematic representation presented at the beginning of the study (Fig. 1.2), we can add the potential research not studied in this work (*grey areas*, Fig. 8.1). First, the effects of the identified threats to *Polylepis* woodlands may be studied on the patterns and processes studied for this Ph.D.: global warming may affect the species diversity of *Polylepis* woodlands (*e.g*. Walker *et al.* 2006), or the exotic species may modify the genetic structure of *Polylepis* woodlands through a reduction in gene flow due to the large size of the exotic *Pinus* and *Eucalyptus* trees, that may diminish pollen flow. Second, the effects of the threats may be studied on other patterns and processes not studied in this research, such as the patterns of species evenness, dispersal, or population age structure, or the processes of natural selection, pollination, competition, exploitation, and mutualism.

APPENDICES

Appendix 1. Species list of plants inhabiting the Polylepis woodland remnants in Bolivia.

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Bryophyta				
Bartramiaceae	*Bartramia potosica*	Moss		4
Brachytheciaceae	*Brachythecium sp. IH 12*	Moss		4
Bryaceae	*Bryum sp. IH 5*	Moss		4
Dicranaceae	*Cynodontium sp. IH 10*	Moss		4
Dicranaceae	*Dicranum fugidum*	Moss		4
Grimmiaceae	*Grimmia sp. IH 4*	Moss		4
Hedwigiaceae	*Braunia cirrhifolia*	Moss		4
Neckeraceae	*Neckera sp. IH 9*	Moss		4
Polytrichaceae	*Polytrichum juniperinum*	Moss		4
Pottiaceae	*Leptodontium viticulosoides*	Moss		4
Pottiaceae	*Tortula ruralis*	Moss		4
Thuidiaceae	*Thuidium sp. IH 14*	Moss		4
Pteridophyta				
Pteridaceae	*Adiantum aff. lorentzii*	Fern		3
Pteridaceae	*Adiantum hirsutum*	Fern		3
Pteridaceae	*Adiantum poiretii*	Fern		4
Pteridaceae	*Adiantum sp. EF 290*	Fern		3
Pteridaceae	*Adiantum sp. MR2*	Fern		1
Pteridaceae	*Adiantum thalictroides*	Fern		3
Aspleniaceae	*Asplenium castaneum*	Fern		3,4
Aspleniaceae	*Asplenium cf. lorentzii*	Fern		3,5
Aspleniaceae	*Asplenium cuspidatum*	Fern		3,5
Aspleniaceae	*Asplenium fragile*	Fern		4
Aspleniaceae	*Asplenium gilliesii*	Fern		3
Aspleniaceae	*Asplenium monanthes*	Fern		3,4
Blechnaceae	*Blechnum aff. tabulare*	Fern		3
Blechnaceae	*Blechnum binervatum*	Fern		5
Blechnaceae	*Blechnum buchtienii*	Fern		5
Blechnaceae	*Blechnum cf. cordatum*	Fern		5
Blechnaceae	*Blechnum penna-marina*	Fern		3-5
Blechnaceae	*Blechnum sp.1 EF 428*	Fern		3
Blechnaceae	*Blechnum sprucei*	Fern		3
Polypodiaceae	*Campyloneurum amphostenon*	Fern		3
Polypodiaceae	*Campyloneurum aglaolepis [C. angustifolium]*	Fern		4
Polypodiaceae	*Campyloneurum angustipaleatum*	Fern		3
Polypodiaceae	*Campyloneurum asplundii*	Fern		3
Pteridaceae	*Cheilanthes myriophylla*	Fern		3
Pteridaceae	*Cheilanthes pruinata*	Fern		1,3,4
Cyatheaceae	*Cyathea sp. MM16*	NR		5
Dryopteridaceae	*Cystopteris fragilis*	Fern		4
Dennstaedtiaceae	*Dennstaedtia sp. MM17*	Fern		5
Dryopteridaceae	*Dryopteris cf. paleaceae*	Fern		2,3,5
Dryopteridaceae	*Dryopteris sp.1 EF 174*	Fern		3
Dryopteridaceae	*Dryopteris sp.2 EF 432*	Fern		3
Lomariopsidaceae	*Elaphoglossum sp. EF 594*	Fern		3
Lomariopsidaceae	*Elaphoglossum sp. IH 13*	Fern		4
Lomariopsidaceae	*Elaphoglossum sp. MM4*	Fern		5
Lomariopsidaceae	*Elaphoglossum sp.1 EF 510*	Fern		3
Lomariopsidaceae	*Elaphoglossum sp.3 EF 640*	Fern		3
Lomariopsidaceae	*Elaphoglossum sp.4 EF 475*	Fern		3
Lomariopsidaceae	*Elaphoglossum sp.5 EF 513,633*	Fern		3
Grammitidaceae	*Lellingeria sp. EF 632*	Fern		3

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Refe-rence[d]
Grammitidaceae	*Melpomene moniliformis*	Fern		3
Ophioglossaceae	*Ophioglossum scariosum*	Fern		4
Pteridaceae	*Pellaea ternifolia*	Fern		3,4
Polypodiaceae	*Polypodium aff. latipes*	Fern		2,3
Polypodiaceae	*Polypodium buchtienii*	Fern		4
Polypodiaceae	*Polypodium megalolepis*	Fern		3
Polypodiaceae	*Polypodium pycnophyllum*	Fern		5
Polypodiaceae	*Polypodium pycnocarpum*	Fern		3
Polypodiaceae	*Polypodium sp. MM15*	Fern		5
Polypodiaceae	*Polypodium sp. MR 9*	Fern		1
Dryopteridaceae	*Polystichum cochleatum*	Fern		4
Dryopteridaceae	*Polystichum sp. EF390,524*	Fern		3
Dryopteridaceae	*Polystichum sp. EF478*	Fern		3
Dryopteridaceae	*Polystichum sp. MM1*	Fern		5
Dryopteridaceae	*Polystichum sp. MM11*	Fern		5
Dennstaedtiaceae	*Saccoloma inaequale*	Fern		3
Grammitidaceae	*Terpsichore longisetosa*	Fern		3
Thelypteridaceae	*Thelypteris sp. EF 433*	Fern		3
Dryopteridaceae	*Woodsia montevidensis*	Fern		3,4
Spermatophyta				
Alstroemeriaceae	*Bomarea brevis*	Herb	LC	5
Alstroemeriaceae	*Bomarea cf. aurantiaca*	Herb		3,5,8
Alstroemeriaceae	*Bomarea cf. dulcis*	Herb		2-4,8
Alstroemeriaceae	*Bomarea crocea*	Herb		3,8
Amaranthaceae	*Gomphrena bicolor*	Herb		3,4
Amaranthaceae	*Gomphrena sp. IH 2322*	Herb		4
Anacardiaceae	*Lithrea molleoides [L. terniflora]*	Shrub		8
Anacardiaceae	*Schinus microphyllus [S. andinus]*	Tree		3,4,8
Anacardiaceae	*Schinus myrtifolia*	Shrub		4
Anacardiaceae	*Schinus sp. MR 9*	NR		1
Apiaceae	*Azorella biloba*	Herb		3
Apiaceae	*Azorella compacta*	Herb	V	8
Apiaceae	*Bowlesia flabilis*	Herb		4
Apiaceae	*Bowlesia lobata*	Herb		3
Apiaceae	*Bowlesia sp. MM 14*	Herb		5
Apiaceae	*Bowlesia tenella*	Herb		4
Apiaceae	*Bowlesia tropaeolifolia*	Herb		4
Apiaceae	*Eryngium nudicaule*	Herb		3
Apiaceae	*Eryngium paniculatum*	Herb		1
Apiaceae	*Hydrocotyle indecora [H. bonplandii]*	Herb		4
Apiaceae	*Hydrocotyle sp. MM2*	Herb		5
Apiaceae	*Oreomyrrhis andicola*	Herb		4
Araliaceae	*Oreopanax kuntzei*	Tree		5
Araliaceae	*Oreopanax macrocephalus*	Tree		5
Araliaceae	*Oreopanax rusbyi*	Tree		3,5
Araliaceae	*Oreopanax sp. IH 1*	Tree		4
Araliaceae	*Oreopanax trollii*	Tree		5
Asclepiadaceae	*Sarcostemma campanulatum*	Climber		3
Asclepiadaceae	*Sarcostemma lysimachioides*	Herb		3,4
Asteraceae	*Achryrocline ramosissima*	Herb		3,4
Asteraceae	*Achyrocline alata*	Herb		3,4
Asteraceae	*Achyrocline flaccida*	Herb		3
Asteraceae	*Achyrocline satureioides*	Shrub		3
Asteraceae	*Achyrocline sp. MR 1*	NR		1
Asteraceae	*Achyrocline venosa*	Herb		4
Asteraceae	*Ageratina azangoroensis*	Herb		3,4
Asteraceae	*Ageratina sternbergiana*	Herb		3,4

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Refe-rence[d]
Asteraceae	*Baccharis alpina*	Herb		3,4
Asteraceae	*Baccharis cassinifolia*	Tree		4
Asteraceae	*Baccharis dracunculifolia*	Shrub		1,3,4
Asteraceae	*Baccharis latifolia*	Shrub		4,5
Asteraceae	*Baccharis leptophylla*	Shrub		4
Asteraceae	*Baccharis nitida*	Tree		3-5,8
Asteraceae	*Baccharis oblongifolia*	Shrub		5
Asteraceae	*Baccharis obtusifolia*	Shrub		1,3,4
Asteraceae	*Baccharis papillosa*	Shrub		8
Asteraceae	*Baccharis pentlandii*	Shrub		3,5,8
Asteraceae	*Baccharis peruviana*	Shrub		2
Asteraceae	*Baccharis pflanzii*	Shrub		3
Asteraceae	*Baccharis rufescens [B. polycephala]*	Shrub		3,8
Asteraceae	*Baccharis sp. IH 19*	NR		4
Asteraceae	*Baccharis subalata*	Shrub		3-5
Asteraceae	*Baccharis tricuneata*	Shrub		4
Asteraceae	*Baccharis trimera [B. genistelloides]*	Subshrub		3-5,8
Asteraceae	*Baccharis volubilis*	Climber-leaner		3
Asteraceae	*Baccharis yungensis*	Tree		8
Asteraceae	*Barnadesia berberoides*	Shrub		3
Asteraceae	*Barnadesia odorata*	Shrub		8
Asteraceae	*Barnadesia polyacantha*	Shrub		4,5
Asteraceae	*Barnadesia pycnophylla*	Shrub		4
Asteraceae	*Belloa pickeringii*	Herb		3
Asteraceae	*Bidens andicola*	Herb		1,3,4
Asteraceae	*Bidens pilosa*	Herb		4
Asteraceae	*Bidens pseudocosmos*	Herb		3,4
Asteraceae	*Coniza deserticola*	Herb		4
Asteraceae	*Coniza floribunda*	Herb		4
Asteraceae	*Coniza sp. MF2*	Herb		2
Asteraceae	*Coniza spiciformis*	Herb		4
Asteraceae	*Coreopsis fasciculata*	Herb		4
Asteraceae	*Cosmos peucedanifolius*	Herb		1-4
Asteraceae	*Dasyphyllum brasiliense*	Shrub		8
Asteraceae	*Dasyphyllum hystrix*	Shrub		8
Asteraceae	*Eupatorium buniifolium*	Shrub		4,8
Asteraceae	*Eupatorium sp. IH 2468*	NR		4
Asteraceae	*Facelis lasiocarpa*	Herb		4
Asteraceae	*Galinsoga mandonii*	Herb		4
Asteraceae	*Gamochaeta americana [G. spicata]*	Herb		4
Asteraceae	*Gamochaeta simplicaulis*	Herb		4
Asteraceae	*Gamochaeta sp. MR 4*	Herb		1
Asteraceae	*Gamochaeta sphacelata*	Herb		3,4
Asteraceae	*Gnaphalium cheiranthifolium*	Herb		4
Asteraceae	*Gnaphalium dombeyanum*	Herb		1
Asteraceae	*Gnaphalium gaudichaudianum*	Herb		3,4
Asteraceae	*Gynoxys asterotricha*	Tree		8
Asteraceae	*Gynoxys psilophylla [G. glabriuscula]*	Shrub		2-5,8
Asteraceae	*Gynoxys sp. MM6*	NR		5
Asteraceae	*Hieracium aff. adenocephalum [H. streptochaetum]*	Herb		3,4
Asteraceae	*Hieracium lagopus*	Herb		3
Asteraceae	*Hieracium mandonii*	Herb		4
Asteraceae	*Hieracium microcephalum*	Herb		3
Asteraceae	*Hieracium stachyoideum*	Herb		4
Asteraceae	*Hypochaeris chillensis*	Herb		3

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Asteraceae	*Hypochaeris elata*	Herb		4
Asteraceae	*Hypochaeris meyeniana*	Herb		4
Asteraceae	*Jungia herzogiana*	Herb		4
Asteraceae	*Liabum ovatum*	Herb		4
Asteraceae	*Mutisia lanigera*	Herb		8
Asteraceae	*Mutisia ledifolia*	Shrub		8
Asteraceae	*Mutisia mandoniana*	Shrub		3,4
Asteraceae	*Mutisia matthewsii*	Shrub		4
Asteraceae	*Mutisia orbignyana*	Shrub		8
Asteraceae	*Mutisia vicia*	Shrub		4
Asteraceae	*Noticastrum marginatum*	Herb		4
Asteraceae	*Ophryosporus charua*	Shrub		4
Asteraceae	*Ophryosporus heptanthus*	Shrub		2
Asteraceae	*Parastrephia lepidophylla*	Shrub		8
Asteraceae	*Parastrephia lucida*	Shrub		8
Asteraceae	*Parastrephia quadrangularis*	Shrub	V	8
Asteraceae	*Perezia pungens*	Herb		3
Asteraceae	*Plazia daphnoides*	Shrub		3,4
Asteraceae	*Senecio af. epiphyticus*	Shrub		5
Asteraceae	*Senecio clivicolus*	Herb		1,4
Asteraceae	*Senecio oronocensis*	Herb		5
Asteraceae	*Senecio sp. EF 553*	NR		3
Asteraceae	*Senecio sp. IH 1335*	NR		4
Asteraceae	*Senecio sp. MF 7*	NR		2
Asteraceae	*Stevia chamaedrys*	Herb		3,4
Asteraceae	*Stevia mercedensis*	Herb		4
Asteraceae	*Stevia neglecta*	Herb		4
Asteraceae	*Stevia samaipatensis*	Herb		1,3,4
Asteraceae	*Stevia stuebelii*	Herb		4
Asteraceae	*Stevia tarijensis*	Herb		4
Asteraceae	*Tagetes maxima*	Shrub		4
Asteraceae	*Tagetes multiflora*	Herb		4
Asteraceae	*Tagetes pusilla*	Herb		4
Asteraceae	*Taraxacum officinale*	Herb		3
Asteraceae	*Verbesina cinerea*	Herb		3
Asteraceae	*Viguiera aff. fusiformis*	Subshrub		3
Asteraceae	*Viguiera pazensis*	Subshrub		4
Asteraceae	*Viguiera procumbens*	Subshrub		1,3,4
Begoniaceae	*Begonia aff. baumannii*	Herb		3
Begoniaceae	*Begonia aff. veitchii*	Herb		3
Begoniaceae	*Begonia sp. IH 8*	NR		4
Berberidaceae	*Berberis boliviana*	Shrub		4
Berberidaceae	*Berberis bumeliaefolia*	Shrub		8
Berberidaceae	*Berberis cf. argentinensis*	Shrub		4
Berberidaceae	*Berberis cf. rariflora*	Shrub		1,3-5,8
Berberidaceae	*Berberis chrysacantha*	Shrub		8
Berberidaceae	*Berberis commutata*	Shrub		1-5,8
Berberidaceae	*Berberis sp. IH 1021*	NR		4
Berberidaceae	*Berberis sp. IH 1073*	NR		4
Berberidaceae	*Berberis paucidentata*	Shrub		2,3,8
Berberidaceae	*Berberis sp. IH 2425*	NR		4
Berberidaceae	*Berberis weddellii*	Shrub		3,5
Betulaceae	*Alnus acuminata*	Tree		3,4,8
Brassicaceae	*Cardamine ovata*	Herb		5
Bromeliaceae	*Puya glabrescens*	Succulent		3
Bromeliaceae	*Puya herzogii*	Succulent		3
Bromeliaceae	*Puya sp. IH S*	Succulent		4

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Bromeliaceae	*Puya sp. MR 8*	Succulent		1
Bromeliaceae	*Puya tunarensis*	Succulent		1,3
Bromeliaceae	*Tillandsia capitata*	Epyphite		4
Bromeliaceae	*Tillandsia recurvata*	Epyphite		3
Bromeliaceae	*Tillandsia usneoides*	Epyphite		1,4
Bromeliaceae	*Tillandsia virescens*	Epyphite		7
Brunelliaceae	*Brunellia sp. RM1*	Tree		6
Buddlejaceae	*Buddleja aromatica*	Tree		8
Cactaceae	Cactaceae sp. IH 16	Succulent		4
Cactaceae	*Echinopsis obrepanda*	Succulent		3
Cactaceae	*Echinopsis tarijensis*	Succulent		8
Cactaceae	*Lobivia maximiliana*	Succulent		3
Cactaceae	*Opuntia chuquisacana*	Succulent		3
Cactaceae	*Opuntia sulphurea*	Succulent		3
Cactaceae	*Sulcorebutia steinbachii*	Succulent	V	3
Cactaceae	*Trichocereus cf. taratensis*	Succulent		8
Cactaceae	*Trichocereus sp. IH 15*	Succulent		4
Cactaceae	*Echinopsis tunariensis*	Succulent	V	1,3
Campanulaceae	*Lobelia nana*	Herb		3
Campanulaceae	*Siphocampylus tunarensis*	Subshrub		5
Campanulaceae	*Wahlenbergia linarioides*	Herb		4
Caryophyllaceae	*Arenaria lanuginosa*	Herb		3
Caryophyllaceae	*Arenaria sp. IH 2316*	NR		4
Caryophyllaceae	*Cerastium arvense*	Herb		3
Caryophyllaceae	*Cerastium mucronatum*	Herb		4
Caryophyllaceae	*Cerastium nutans*	Herb		4
Caryophyllaceae	*Cerastium sp. IH 1045*	Herb		4
Caryophyllaceae	*Paronychia hieronymi*	Herb		4
Caryophyllaceae	*Silene genovevae*	Herb		3,4
Caryophyllaceae	*Spergularia sp. IH 19*	Herb		4
Caryophyllaceae	*Stellaria sp. MM15*	Herb		5
Caryophyllaceae	*Stellaria weddellii*	Herb		3
Celastraceae	*Maytenus verticillata*	Tree		3,8
Clethraceae	*Clethra cuneata*	Tree		3,5
Clethraceae	*Clethra sp. IH 7*	Tree		4
Commelinaceae	*Commelina elliptica*	Herb		3
Convolvulaceae	*Dichondra argentea*	Herb		4
Convolvulaceae	*Dichondra sericea*	Herb		3,4
Convolvulaceae	*Evolvulus sericeus*	Herb		4
Cunoniaceae	*Weinmannia fagaroides*	Tree	V	3-5
Cunoniaceae	*Weinmannia microphylla*	Tree		5
Cunoniaceae	*Weinmannia sp. RM2*	Tree		6
Cuscutaceae	*Cuscuta cf. odorata*	Parasite		3
Cyperaceae	*Bulbostylis capillaris*	Grass		4
Cyperaceae	*Bulbostylis cf. juncoides*	Grass		3
Cyperaceae	*Bulbostylis sp. IH 17*	Grass		4
Cyperaceae	*Carex acaule*	Grass		3
Cyperaceae	*Carex sp. IH 2*	Grass		4
Cyperaceae	*Cyperus andinus*	Grass		4
Cyperaceae	*Cyperus sp. MR 3*	Grass		1
Cyperaceae	*Rhynchospora cf. hieronymi*	Grass		5
Cyperaceae	*Uncinia phleoides*	Grass		3
Elaeocarpaceae	*Vallea stipularis*	Tree		3-5,8
Ephedraecae	*Ephedra americana*	Shrub		3
Ephedraecae	*Ephedra rupestris*	Shrub		3
Ericaceae	*Gaultheria bracteata*	Shrub		5
Ericaceae	*Gaultheria glomerata*	Shrub		3-5

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Ericaceae	*Pernettya prostrata*	Subshrub		3-5
Ericaceae	*Polyclita turbinata*	Shrub		3,5
Ericaceae	*Vaccinium floribundum*	Shrub		2,4
Escalloniaceae	*Escallonia hypoglauca*	Tree		8
Escalloniaceae	*Escallonia myrtilloides*	Tree	LC	3,8
Escalloniaceae	*Escallonia patens*	Tree		3,5
Escalloniaceae	*Escallonia resinosa*	Tree	V	3,4,8
Escalloniaceae	*Escallonia schreiteri*	Tree		8
Fabaceae	*Adesmia miraflorensis*	Shrub		3
Fabaceae	*Adesmia sp. IH 2421*	NR		4
Fabaceae	*Amicia andicola*	Herb		4
Fabaceae	*Amicia fimbriata*	Herb		3
Fabaceae	*Astragalus sp. EF 364*	NR		3
Fabaceae	*Cologania broussonetii*	Herb		3
Fabaceae	*Dalea boliviana*	Herb		3
Fabaceae	*Dalea tarapacana*	NR		4
Fabaceae	*Lathyrus magellanicus*	Subshrub		3,4
Fabaceae	*Lupinus altimontanus*	Subshrub		3
Fabaceae	*Lupinus sp. MR 5*	NR		1
Fabaceae	*Senna aymara*	Shrub		4
Fabaceae	*Trifolium amabile*	Herb		1,3,4
Fabaceae	*Vicia graminea*	Herb		3
Fabaceae	*Vicia setifolia*	Herb		4
Flacourtiaceae	*Azara salicifolia*	Shrub		3,5,8
Gentianaceae	*Gentiana sedifolia*	Herb		4
Gentianaceae	*Gentianella dielsiana*	Herb		4
Gentianaceae	*Gentianella hypssopifolia*	Herb		3
Gentianaceae	*Gentianella larecajensis*	Herb		2
Gentianaceae	*Gentianella neomandonii*	Herb		3
Gentianaceae	*Gentianella sp. 1 EF 360*	Herb		3
Gentianaceae	*Gentianella sp. 2 EF 458*	Herb		3
Geraniaceae	*Erodium moschatum*	Grass		4
Geraniaceae	*Geranium cf. bolivianum*	Herb		3,4
Geraniaceae	*Geranium cf. fiebrigii*	Herb		4
Geraniaceae	*Geranium cf. sessiliflorum*	Herb		3,4
Geraniaceae	*Geranium cf. velutinum*	Herb		4
Geraniaceae	*Geranium soratae*	Herb		3
Geraniaceae	*Geranium sp. MF1*	Herb		2
Grossulariaceae	*Ribes cf. brachybotrys*	Shrub		2-5,8
Grossulariaceae	*Ribes glandulosum*	Shrub		5
Grossulariaceae	*Ribes sp. IH 1063*	Shrub		4
Grossulariaceae	*Ribes sp. IH 1743*	Shrub		4
Gunneraceae	*Gunnera cf. scabra*	Herb		5
Gunneraceae	*Gunnera magellanica*	Herb		3
Gunneraceae	*Gunnera sp. EF 440*	Herb		3
Hydrophyllaceae	*Phacelia secunda*	Herb		4
Hypericaceae	*Hypericum brevistylum*	Herb		3,4
Iridaceae	*Trifurcia lahue [Alophia lahue]*	Grass		3
Iridaceae	*Olsynium sp. MF 5*	Grass		2
Iridaceae	*Orthrosanthus chimboracensis*	Grass		2-4
Iridaceae	*Sisyrinchium cf. chilense*	Grass		4
Iridaceae	*Sisyrinchium jamesoni*	Grass		3
Iridaceae	*Sisyrinchium aff. junceum [Olsynium aff. junceum]*	Grass		3,4
Iridaceae	*Sisyrinchium pachyrhizum*	Grass		4
Iridaceae	*Sisyrinchium palustre*	Grass		3
Iridaceae	*Sisyrinchium sp. EF 468*	Grass		3

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Refe-rence[d]
Iridaceae	*Sisyrinchium trinerve*	Grass		4
Juncaceae	*Juncus sp. IH 1957*	Grass		4
Juncaceae	*Luzula excelsa*	Grass		3,4
Juncaceae	*Luzula racemosa*	Grass		3,4
Juncaceae	*Luzula sp. MR 6*	Grass		1
Lamiaceae	*Hedeoma mandoniana*	Herb		3
Lamiaceae	*Hedeoma mandonii*	Herb		4
Lamiaceae	*Lepechinia bella*	Shrub		4
Lamiaceae	*Lepechinia graveolens*	Shrub		4
Lamiaceae	*Lepechinia meyenii*	Herb		1,3,4
Lamiaceae	*Minthostachys andina*	Shrub		3,4
Lamiaceae	*Minthostachys sp. IH 13*	NR		4
Lamiaceae	*Minthostachys sp. MM 18*	NR		3
Lamiaceae	*Salvia haenkei*	Subshrub		3,4
Lamiaceae	*Salvia sp. IH 2008*	NR		4
Lamiaceae	*Clinopodium bolivianum [Satureja boliviana]*	Shrub		1,3-5,8
Liliaceae	*Nothoscordum andicola*	Herb		3
Liliaceae	*Nothoscordum bivalve*	Herb		3
Loasaceae	*Caiophora canarinoides*	Herb		3-5
Loasaceae	*Caiophora horrida*	Herb		1,3
Loranthaceae	*Gaiadendron punctatum*	Tree		5
Loranthaceae	*Tristerix penduliflorus*	Parasite		1,3,4,8
Lycopodiaceae	*Lycopodium clavatum*	Herb		3,4
Lycopodiaceae	*Lycopodium thyoides [L. complanatum]*	Herb		3,4
Lythraceae	*Cuphea cf. campyloneuron*	Herb		4
Malvaceae	*Tarasa hornschuchiana*	Shrub		3
Melastomataceae	*Brachyotum microdon*	Shrub		3-5,8
Melastomataceae	Melastomataceae sp. IH 1798	NR		4
Melastomataceae	*Miconia cf. mandonii*	Shrub		5
Melastomataceae	*Miconia sp. MM5*	NR		5
Myricaceae	*Myrica pubescens*	Tree		3,5,8
Myrsinaceae	*Myrsine aff. pearcei*	Tree		5
Myrsinaceae	*Myrsine sp. EF 531*	NR		3
Myrtaceae	*Myrcianthes pseudomato*	Tree		8
Myrtaceae	*Myrteola phylicoides*	Tree		5
Onagraceae	*Fuchsia apetala*	Herb		2,3,5,8
Onagraceae	*Fuchsia nana*	Herb		3,5
Onagraceae	*Oenothera versicolor*	Herb		3
Orchidaceae	*Myrosmodes paludosum [Aa paludosa]*	Herb		3
Orchidaceae	*Habenaria pumiloides*	Herb		3
Orchidaceae	*Malaxis sp. IH 11*	Herb		4
Orchidaceae	*Malaxis sp. MM12*	Herb		5
Orchidaceae	*Malaxis sp. 1 EF 310*	Herb		3
Orchidaceae	*Malaxis sp. 2 EF 423*	Herb		3
Oxalidaceae	*Hypseocharis pimpinellifolius*	Herb		3
Oxalidaceae	*Hypseocharis sp. IH3*	Herb		4
Oxalidaceae	*Oxalis cf. epicardica*	Herb		3
Oxalidaceae	*Oxalis cf. mollissima*	Herb		4
Oxalidaceae	*Oxalis corniculata*	Herb		3,5
Oxalidaceae	*Oxalis lotoides*	Herb		3,4
Oxalidaceae	*Oxalis sp. IH 2078*	Herb		4
Oxalidaceae	*Oxalis sp. IH S*	Herb		4
Oxalidaceae	*Oxalis violacea*	Herb		3
Passifloraceae	*Passiflora exoperculata*	Climber-leaner		3
Passifloraceae	*Passiflora mandonii*	Herb		8

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Passifloraceae	*Passiflora pinnatistipula*	Climber-leaner		3
Passifloraceae	*Passiflora sp. MR 7*	Climber-leaner		1
Piperaceae	*Peperomia cyclaminoides*	Herb		3
Piperaceae	*Peperomia fiebrigii*	Herb		3-5
Piperaceae	*Peperomia galioides*	Herb		4,5
Piperaceae	*Peperomia peruviana*	Herb		3
Plantaginaceae	*Plantago orbignyana*	Herb		3,4
Plantaginaceae	*Plantago tomentosa*	Herb		4
Poaceae	*Aristida adscensionis*	Grass		3,4,8
Poaceae	*Briza monandra*	Grass		4
Poaceae	*Briza stricta*	Grass		4
Poaceae	*Bromus catharticus*	Grass		4
Poaceae	*Bromus lanatus*	Grass		3,4
Poaceae	*Bromus pitensis*	Grass		4
Poaceae	*Bromus trinii*	Grass		4
Poaceae	*Bromus unioloides*	Grass		2
Poaceae	*Calamagrostis amoena [Deyeuxia amoena]*	Grass		4
Poaceae	*Calamagrostis antoniana*	Grass		2-4,8
Poaceae	*Calamagrostis breviaristata*	Grass		4
Poaceae	*Calamagrostis curvula [Deyeuxia curvula]*	Grass		3
Poaceae	*Calamagrostis heterophylla*	Grass		3
Poaceae	*Calamagrostis intermedia*	Grass		4
Poaceae	*Calamagrostis sp. MF4*	Grass		2
Poaceae	*Calamagrostis sp. MR3*	Grass		1
Poaceae	*Calamagrostis tarmensis*	Grass		3,8
Poaceae	*Calamagrostis vicunarum*	Grass		3
Poaceae	*Chascolytrum subaristatum*	Grass		3
Poaceae	*Chusquea sp.MM10*	NR		5
Poaceae	*Elionurus muticus*	Grass		4
Poaceae	*Elionurus tripsacoides*	Grass		3,8
Poaceae	*Eragrostis sp. IH 20*	Grass		4
Poaceae	*Festuca gr. dolicophylla*	Grass		1-4,8
Poaceae	*Festuca orthophylla*	Grass		8
Poaceae	*Festuca sp. MM7*	Grass		5
Poaceae	*Festuca sublimis*	Grass		3,4
Poaceae	*Graminea sp. MM9*	Grass		5
Poaceae	*Lycurus phleoides*	Grass		4
Poaceae	*Muhlenbergia peruviana*	Grass		3
Poaceae	*Muhlenbergia rigida*	Grass		4
Poaceae	*Nassella brachyphylla*	Grass		3
Poaceae	*Nassella sp. IH 1712*	Grass		4
Poaceae	*Nassella inconspicua*	Grass		3
Poaceae	*Nassella mucronata*	Grass		3
Poaceae	*Nassella publiflora*	Grass		4
Poaceae	*Piptochaetium montevidense*	Grass		3
Poaceae	*Piptochaetium panicoides*	Grass		4
Poaceae	*Piptochaetium tuberculatum*	Grass		4
Poaceae	*Poa annua*	Grass		4
Poaceae	*Poa buchtienii*	Grass		3
Poaceae	*Poa horridula*	Grass		4
Poaceae	*Sorghastrum stipoides*	Grass		3
Poaceae	*Stipa brachyphylla*	Grass		4
Poaceae	*Stipa ichu*	Grass		1,3,4
Poaceae	*Stipa mucronata*	Grass		4

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Poaceae	*Stipa sp.1 EF 585*	Grass		3
Poaceae	*Stipa sp.2 EF 573*	Grass		3
Poaceae	*Trisetum spicatum*	Grass		3
Poaceae	*Vulpia megalura*	Grass		4
Podocarpaceae	*Podocarpus cardenasii [P. glomeratus]*	Tree		5
Podocarpaceae	*Podocarpus parlatorei*	Tree	LC	8
Podocarpaceae	*Prumnopitys exigua*	Tree	V	3-5
Polemoniaceae	*Gilia lacinata*	Herb		4
Polygalaceae	*Monnina bridgesii*	Shrub		5
Polygalaceae	*Monnina eriocarpa*	Herb		4
Polygalaceae	*Monnina salicifolia*	Subshrub		3,4
Polygalaceae	*Polygala cisandina*	Herb		4
Polygonaceae	*Muehlenbeckia tamnifolia*	Climber		3
Polygonaceae	*Muehlenbeckia volcanica*	Herb		3
Polygonaceae	*Rumex acetosella*	Herb		3
Pottiaceae	*Barbula sp. IH 18*	NR		4
Ranunculaceae	*Anemone decapetala*	Herb		3
Ranunculaceae	*Clematis sericea*	Climber-leaner		4
Ranunculaceae	*Thalictrum decipiens*	Herb		8
Ranunculaceae	*Thalictrum venturii*	Herb		4
Rhamnaceae	*Colletia spinosissima*	Shrub		3,8
Rhamnaceae	*Kentrothamnus weddellianus*	Shrub		8
Rosaceae	*Acaena cylindristachya*	Herb		3
Rosaceae	*Alchemilla aphanoides [Lachemilla aphanoides]*	Herb		2,4
Rosaceae	*Hesperomeles cuneata*	Tree		3,4
Rosaceae	*Hesperomeles lanuginosa*	Tree		3-5,8
Rosaceae	*Hesperomeles pernettyoides*	Tree		3,5
Rosaceae	*Kageneckia lanceolata*	Tree		3,8
Rosaceae	*Lachemilla pinnata [Alchemilla pinnata]*	Herb		3,4
Rosaceae	*Margyricarpus setosus [M. pinnatus]*	Shrub		4
Rosaceae	*Prunus tucumanensis*	Tree		3,8
Rosaceae	*Rubus nubigenus*	Shrub		3
Rosaceae	*Tetraglochin cristatum*	Subshrub		3
Rubiaceae	*Borreria densiflora*	Herb		4
Rubiaceae	*Galium corymbosum*	Herb		3
Rubiaceae	*Galium gracilicaule*	Herb		2
Rubiaceae	*Galium hypocarpium*	Herb		3
Rubiaceae	*Galium mandonii*	Herb		4
Rubiaceae	*Galium obovatum*	Herb		4
Rubiaceae	*Galium ovata*	Herb		5
Rubiaceae	*Galium sp. MM13*	Herb		5
Rubiaceae	*Relbunium sp. IH S*	Herb		4
Rubiaceae	*Richardia coldenioides*	Herb		3
Rubiaceae	*Richardia humistrata*	Herb		3
Rubiaceae	*Richardia stellaris*	Herb		4
Rutaceae	*Zanthoxylum coco*	Tree		8
Santalaceae	*Quinchamalium procumbens*	Herb		4
Sapindaceae	*Dodonaea viscosa*	Shrub		3,4,6,8
Scrophulariaceae	*Agalinis bangii*	Herb		3
Scrophulariaceae	*Agalinis reflexidens*	Herb		4
Scrophulariaceae	*Alonsoa acutifolia*	Herb		3
Scrophulariaceae	*Alonsoa linearis*	Herb		4
Scrophulariaceae	*Bartsia crenata*	Herb		2-4
Scrophulariaceae	*Bartsia elongata*	Herb		4
Scrophulariaceae	*Bartsia fiebrigii*	Herb		4

Group or Family	Species[a]	Life form[b]	Threatened status[c]	Reference[d]
Scrophulariaceae	*Calceolaria aquatica*	Herb		3
Scrophulariaceae	*Calceolaria bartsiaefolia*	Herb		3
Scrophulariaceae	*Calceolaria engleriana*	Herb		3,4
Scrophulariaceae	*Calceolaria parvifolia*	Herb		3,8
Scrophulariaceae	*Calceolaria schickendantziana*	Herb		4
Scrophulariaceae	*Calceolaria sp. MF3*	Herb		2
Scrophulariaceae	*Calceolaria sp. MM8*	Herb		5
Scrophulariaceae	*Calceolaria sparsiflora*	Herb		4
Scrophulariaceae	*Calceolaris lobata*	Herb		4
Scrophulariaceae	*Castilleja pumila*	Herb		4
Selaginellaceae	*Selaginella sp. IH 21*	NR		4
Solanaceae	*Dunalia brachyacantha*	Shrub		3,8
Solanaceae	*Salpichroa glandulosa*	Shrub		2,3,8
Solanaceae	*Salpichroa tristis*	Shrub		3,4,8
Solanaceae	*Saracha punctata*	Shrub		3
Solanaceae	*Solanum acaule*	Herb		4
Solanaceae	*Solanum cf. atricoeruleum*	Herb		4
Solanaceae	*Solanum cochabambense*	Shrub		4
Solanaceae	*Solanum maturecalvans*	Shrub		5
Solanaceae	*Solanum mutans*	NR		5
Solanaceae	*Solanum sp. 2 EF 207*	NR		3
Solanaceae	*Solanum sp. EF 173*	NR		3
Solanaceae	*Solanum sp. IH 1338*	NR		4
Solanaceae	*Solanum sp. IH 14*	NR		4
Solanaceae	*Solanum sp. MF 8*	NR		2
Solanaceae	*Solanum sp. MM18*	NR		5
Solanaceae	*Solanum sp. MM3*	NR		5
Solanaceae	*Solanum sp. MR 10*	NR		1
Symplocaceae	*Symplocos nana*	Tree		3,5
Symplocaceae	*Symplocos subcuneata*	Tree		3,5
Tropaeolaceae	*Tropaeolum sp. EF 435*	Climber-leaner		3
Urticaceae	*Pilea dauciodora*	Herb		5
Urticaceae	*Urtica chamaedryoides*	Herb		3,4
Urticaceae	*Urtica echinata*	Herb		5
Valerianaceae	*Valeriana cd. effusa*	Herb		4
Valerianaceae	*Valeriana decussata*	Herb		3
Verbenaceae	*Citharexylum punctatum*	Tree		1,3-5,8
Verbenaceae	*Citharexylum sp. RM3*	Tree		6
Verbenaceae	*Duranta serratifolia*	Shrub		8
Verbenaceae	*Verbena berterii*	Herb		3
Verbenaceae	*Junelia minutifolia [Verbena microphylla]*	Herb		4

[a] Species names follow the most recent paper in the review reporting the species. The codes for species are just for differentiating species within and among papers, it was assumed that species with the same genus in different papers were different species. If one species in a paper was reported as *aff. gr.* or *cf.* then this category holds for the species in this list.

[b] NR= Not reported

[c] LC= Least concern, V= Vulnerable, E= Endangered

[d] References:

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2. Fernández M., M. Mercado, S. Arrázola *et al*. (2001) Structure and floral composition of one forest fragment of *Polylepis besseri* Hieron. subsp. *besseri* in Sacha Loma (Cochabamba). *Revista Boliviana de Ecología y Conservación Ambiental* 9:15-27.

3. Fernández-Terrazas E. (1997) Estudio fitosociológico de los bosques de Kewiña (*Polylepis spp.* Rosaceae) en la Cordillera de Cochabamba. *Revista Boliviana de Ecología y Conservación Ambiental* 2:49-65.
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6. Mueller R., S.G. Beck, R. Lara (2002) Potential vegetation based on climate-data in the Bolivian Yungas-forests. *Ecología en Bolivia* 37:5-14.
7. Navarro G. (2001) Contribución al conocimiento fitosociológico de la vegetación de epífitos vasculares del centro sur de Bolivia. *Revista Boliviana de Ecología y Conservación Ambiental* 10:59-79.
8. Navarro G., J.A. Molina, N. De la Barra (2005) Classification of the high-Andean *Polylepis* forests *in* Bolivia. *Plant Ecology* 176:113-130.

Appendix 2. Species list of animals using the *Polylepis* woodland remnants in Bolivia

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Birds				
Accipitridae	*Accipiter bicolor*	C	LC	3,10
Accipitridae	*Accipiter striatus*	C	LC	5,10,11
Accipitridae	*Buteo magnirostris*	C	LC	5,10
Accipitridae	*Buteo poecilochrous*	C	LC	3,5,6,10,11
Accipitridae	*Buteo polyosoma*	C	LC	3,5,6,10-13
Accipitridae	*Circus cinereus*	C	LC	5
Accipitridae	*Geranoaetus melanoleucus*	C	LC	5,10-13
Anatidae	*Merganetta armata*	I	LC	5
Apodidae	*Aeronautes andecolus*	I	LC	5,10-13
Apodidae	*Aeronautes montivagus*	I	LC	13
Apodidae	*Streptoprocne zonaris*	I	LC	5,10,11,13
Caprimulgidae	*Caprimulgus longirostris*	I	LC	3,5,6,9,10-12
Cardinalidae	*Pheucticus aureoventris*	I, FG	LC	5,11
Cardinalidae	*Saltator aurantiirostris*	FG	LC	3,5,6,9-11,13
Cardinalidae	*Saltator rufiventris*	FG	NT	3,5,6,9-11,13
Cathartidae	*Cathartes aura*	C, O	LC	5,11,13
Cathartidae	*Vultur gryphus*	C, O	NT, V	3,5,6,10-13
Charadriidae	*Phegornis mitchelli [mitchellii]*	I	NT	5
Charadriidae	*Vanellus resplendens*	I	LC	This study
Cinclidae	*Cinclus leucocephalus*	I	LC	5,10
Columbidae	*Columba fasciata*	FG	LC	3,5,6,12
Columbidae	*Columba maculosa*	FG	LC	3,5,9,19,13
Columbidae	*Columbina picui*	FG	LC	5
Columbidae	*Leptotila megalura*	FG	LC	11
Columbidae	*Leptotila verreauxi*	FG	LC	5,11
Columbidae	*Metriopelia aymara*	FG	LC	10
Columbidae	*Metriopelia ceciliae*	FG	LC	3,5,6,10,11
Columbidae	*Metriopelia melanoptera*	FG	LC	3,5,6,9,10,11
Columbidae	*Zenaida auriculata*	FG	LC	3,5,6,10
Corvidae	*Cyanolyca viridicyana*	O	LC	12
Cotingidae	*Ampelion rubrocristatus*	FG	LC	3,5,6,9,10,12
Cotingidae	*Phytotoma rutila*	FG	LC	5
Cracidae	*Penelope dabbenei*	FG	LC, V	10,11
Cracidae	*Penelope montagnii*	FG	LC	12
Cuculidae	*Crotophaga ani*	I, O	LC	5
Dendrocolaptidae	*Lepidocolaptes lacrymiger*	I	LC	12
Dendrocolaptidae	*Xiphocolaptes promeropirhynchus*	I	LC	This study
Emberizidae	*Atlapetes fulviceps*	FG	LC	3,5,9,10,11
Emberizidae	*Atlapetes rufinucha*	FG	LC	9,10,12
Emberizidae	*Buarremon torquata [Atlapetes torquatos]*	I	LC	10,12
Emberizidae	*Catamenia analis*	FG	LC	3,5,6,10,12
Emberizidae	*Catamenia homochroa*	I, FG	LC	This study
Emberizidae	*Catamenia inornata*	FG	LC	3,5,6,9-11,12
Emberizidae	*Diuca speculifera*	FG	LC	5,6,10,12
Emberizidae	*Embernagra platensis*	FG	LC	3,5,10
Emberizidae	*Idiopsar brachyurus*	FG	LC	5
Emberizidae	*Lophospingus griseocristatus*	I, FG	LC	This study
Emberizidae	*Phrygilus alaudinus*	FG	LC	5,10
Emberizidae	*Phrygilus atriceps*	FG	LC	3,5,6,9,10,11
Emberizidae	*Phrygilus dorsalis*	FG	LC	10
Emberizidae	*Phrygilus erythronotus*	FG	LC	This study
Emberizidae	*Phrygilus fruticeti*	FG	LC	3,5,9,10
Emberizidae	*Phrygilus plebejus*	FG	LC	5,6,9-13

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Emberizidae	*Phrygilus punensis*	FG	LC	9,10
Emberizidae	*Phrygilus unicolor*	FG	LC	3,5,6,9-13
Emberizidae	*Poospiza boliviana*	FG	LC	5,9,10
Emberizidae	*Poospiza erythrophrys*	FG	LC	3,5,9,11
Emberizidae	*Poospiza garleppi*	FG	E, E	3,5,6,9,10
Emberizidae	*Poospiza hypochondria*	FG	LC	3,5,6,9-11,13
Emberizidae	*Poospiza torquata*	FG	LC	5,10
Emberizidae	*Poospiza whitii*	FG	NR	10
Emberizidae	*Sicalis flaveola*	FG	LC	5,6
Emberizidae	*Sicalis lutea*	FG	LC	5,6
Emberizidae	*Sicalis luteocephala*	FG	LC	5,10
Emberizidae	*Sicalis olivascens*	FG	LC	3,5,6,10
Emberizidae	*Sicalis uropygialis*	FG	LC	5,6,10
Emberizidae	*Sporophila caerulescens*	FG	LC	This study
Emberizidae	*Sporophila nigricollis*	FG	LC	10
Emberizidae	*Zonotrichia capensis*	FG	LC	3,5,6,9-13
Falconidae	*Falco femoralis*	C	LC	3,5,10,11
Falconidae	*Falco peregrinus*	C	LC	3,5,10
Falconidae	*Falco sparverius*	C	LC	5,10
Falconidae	*Phalcoboenus megalopterus*	C	LC	3,5,6,10,11
Formicariidae	*Grallaria andicola [andicolus]*	I, O	LC, V	9,10
Formicariidae	*Grallaria erythrotis*	I, O	LC	10
Formicariidae	*Grallaria rufula*	I, O	LC	10
Formicariidae	*Grallaria squamigera*	I, O	LC	9,10
Fringillidae	*Carduelis atrata*	FG	LC	5,9-13
Fringillidae	*Carduelis crassirostris*	FG	LC	3,5,9,10,13
Fringillidae	*Carduelis magellanica*	FG	LC	3,5,6,9-13
Furnariidae	*Asthenes dorbignyi*	I	LC	3,5,6,9,10,14,16
Furnariidae	*Asthenes heterura*	I	NT	3,5,6,9,10,11
Furnariidae	*Asthenes maculicauda*	I	LC	3,5,10,14
Furnariidae	*Asthenes modesta*	I, FG	LC	5,10,13,16
Furnariidae	*Asthenes sclateri [punensis]*	I	LC	3,5,10-14
Furnariidae	*Asthenes urubambensis*	I	NT, E	9,10
Furnariidae	*Cinclodes (excelsior) aricomae*	I, FG	CR, CR	10
Furnariidae	*Cinclodes atacamensis*	I	LC	3,5,6,10
Furnariidae	*Cinclodes fuscus*	I, FG	LC	3,5,6,10-13
Furnariidae	*Cranioleuca albiceps*	I	LC	10
Furnariidae	*Cranioleuca pyrrhophia*	I	LC	3,5,10,11
Furnariidae	*Furnarius rufus*	I	LC	3,5,10
Furnariidae	*Geositta cunicularia*	I	LC	This study
Furnariidae	*Geositta punensis*	I, FG	LC	10
Furnariidae	*Geositta rufipennis*	I	LC	10
Furnariidae	*Geositta tenuirostris*	I, FG	LC	5,10
Furnariidae	*Leptasthenura aegithaloides*	I	LC	10
Furnariidae	*Leptasthenura andicola*	I	LC	9,10
Furnariidae	*Leptasthenura fuliginiceps*	I	LC	3,5,6,9-13,16
Furnariidae	*Leptasthenura yanacensis*	I	NT, V	3,5-7,9-14,16
Furnariidae	*Margarornis squamiger*	I	LC	12
Furnariidae	*Ochetorhynchus andaecola [Upucerthia]*	I, N	LC	3,5,6,9,10,16
Furnariidae	*Ochetorhynchus ruficauda [Upucerthia, ruficaudus]*	I	LC	3,9,10
Furnariidae	*Phacellodomus maculipectus*	I	LC	10,11
Furnariidae	*Phacellodomus striaticeps*	I	LC	3,5,6,9,10,13,14
Furnariidae	*Schizoeaca harterti*	I	LC	3
Furnariidae	*Synallaxis azarae [superciliaris]*	I	LC	3,10-12
Furnariidae	*Upucerthia jelskii*	I	LC	5,10,13

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Hirundinidae	*Haplochelidon andecola* [*Hirundo, Notiochelidon*]	I	LC	3,5,6,10,12,13
Hirundinidae	*Hirundo rustica*	I	LC	This study
Hirundinidae	*Notiochelidon cyanoleuca [Pygochelidon cyanoleuca]*	I	LC	3,5,6,11,12
Hirundinidae	*Notiochelidon flavipes*	I	LC	9,12
Hirundinidae	*Notiochelidon murina*	I	LC	3,5,9,10,12
Icteridae	*Cacicus chrysopterus*	I	LC	10,11
Icteridae	*Molothrus badius*	I, FG	LC	3,5,6,10
Icteridae	*Molothrus bonariensis*	I, FG	LC	5
Icteridae	*Oreopsar bolivianus*	I, FG	LC	5
Mimidae	*Mimus dorsalis*	I, FG	LC	3,5,6,10
Motacillidae	*Anthus bogotensis*	I, FG	LC	5
Motacillidae	*Anthus correndera*	I, FG	LC	5,6
Motacillidae	*Anthus furcatus*	I, FG	LC	5
Motacillidae	*Anthus hellmayri*	I	LC	This study
Parulidae	*Basileuterus signatus*	I	LC	This study
Parulidae	*Myioborus brunniceps*	I	LC	3,5,6,9,10-12,16
Parulidae	*Myioborus melanocephalus*	I	LC	10
Picidae	*Colaptes melanochloros*	I	LC	3,5,6,9-11,13
Picidae	*Colaptes rupicola*	I, FG	LC	3,5,6,9-13
Picidae	*Picoides lignarius*	I	NR	3,5
Picidae	*Piculus rivolii*	I	LC	12
Picidae	*Picumnus (cirratus) dorbygnyianus*	I	LC	9,10,11
Picidae	*Veniliornis fumigatus*	I	NR	9,10
Psittacidae	*Amazona tucumana*	FG	NT, V	10,11
Psittacidae	*Aratinga acuticaudata*	FG	LC	11
Psittacidae	*Aratinga mitrata*	FG	LC	5,10-13
Psittacidae	*Bolborhynchus lineola*	FG	LC	12
Psittacidae	*Bolborhynchus orbygnesius*	FG	LC	3,5,9,10,12
Psittacidae	*Brotogeris chiriri*	FG	LC	5
Psittacidae	*Forpus xanthopterygius*	FG	LC	10
Psittacidae	*Pionus tumultuosus*	FG	LC	This study
Psittacidae	*Psilopsiagon aurifrons [Bolborhynchus]*	FG	LC	5,6,9,10,13
Psittacidae	*Psilopsiagon aymara [Bolborhynchus]*	FG	LC	3,5,6,9,10,13
Ramphastidae	*Andigena cucullata*	O	LC	12
Rheidae	*Pterocnemia (pennata) tarapacensis [P. pennata]*	O	NT	10
Rhinocryptidae	*Melanopareia maximiliani*	I	LC	3,5,6,10
Rhinocryptidae	*Scytallopus simonsi*	I	NR	3,5,6,12
Rhinocryptidae	*Scytalopus parvirostris*	I	LC	12
Rhinocryptidae	*Scytalopus schulenbergi*	I	LC	12
Rhinocryptidae	*Scytalopus zimmeri*	I	LC	10,11
Scolopacidae	*Gallinago andina*	I, O	LC	5,10
Scolopacidae	*Gallinago jamesoni*	I, O	LC	This study
Strigidae	*Aegolius harrisii*	C	LC	This study
Strigidae	*Asio stygius*	C	LC	10
Strigidae	*Bubo magellanicus [B. virginianus]*	C	LC	5,10,12
Strigidae	*Glaucidium bolivianum*	C	LC	3,5,6,12,13
Strigidae	*Megascops albogularis*	C	LC	This study
Strigidae	*Megascops choliba*	C, I	LC	This study
Strigidae	*Megascops hoyi [Otus]*	C, I	LC	10,11
Thamnophilidae	*Thamnophilus ruficapillus*	I	LC	10,11
Thinocoridae	*Attagis gayi*	I, FG, B	LC	10

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Thinocoridae	*Thinocorus orbignyianus*	I, FG	LC	5,10
Thraupidae	*Anisognathus igneiventris [igniventris]*	FG	LC	9,10,12
Thraupidae	*Buthraupis montana*	FG	LC	This study
Thraupidae	*Chlorospingus ophthalmicus*	FG	LC	3,5,10,12
Thraupidae	*Conirostrum cinereum*	I, FG	LC	3,5,6,9,10,13
Thraupidae	*Conirostrum ferrugineiventre*	I, FG	LC	3,5,9,10,12
Thraupidae	*Conirostrum sitticolor*	I, FG	LC	9,10
Thraupidae	*Diglossa brunneiventre [brunneiventris]*	N	LC	9,10
Thraupidae	*Diglossa carbonaria*	N	LC	3,5,6,9,10,13
Thraupidae	*Diglossa cyanea*	I	LC	12
Thraupidae	*Diglossa mystacalis*	N	LC	3
Thraupidae	*Diglossa sittoides*	N	LC	3,5,6,10
Thraupidae	*Dubusia castaneoventris [Delothraupis]*	I, FG	LC	This study
Thraupidae	*Euphonia chlorotica*	FG	LC	5
Thraupidae	*Euphonia cyanocephala*	FG	LC	This study
Thraupidae	*Hemispingus trifasciatus*	I	LC	This study
Thraupidae	*Oreomanes fraseri*	I	NT, V	3,5-10,12,14,16
Thraupidae	*Pipraeidea melanonota*	FG, I	LC	10,13
Thraupidae	*Piranga flava*	FG, I	LC	5
Thraupidae	*Tangara vassorii*	FG, I	LC	10
Thraupidae	*Thlypopsis ruficeps*	FG, I	LC	9,10
Thraupidae	*Thraupis bonariensis*	FG, I	LC	3,5,6,9,10,12
Thraupidae	*Thraupis cyanocephala*	FG, I	LC	This study
Thraupidae	*Thraupis sayaca*	FG, I	LC	5,10,11
Tinamidae	*Nothoprocta ornata*	FG	LC	3,5,9,10,13
Tinamidae	*Nothoprocta pentlandii*	I, FG	LC	3,5,6,10
Tinamidae	*Nothura darwinii*	I, FG	LC	3,5,6,10,13
Tinamidae	*Rhynchotus maculicollis*	O	LC	3,11
Tinamidae	*Tinamotis pentlandii*	FG, I	LC	10
Trochilidae	*Amazilia chionogaster*	N	LC	This study
Trochilidae	*Chalcostigma olivaceum*	I, N	LC	9,10
Trochilidae	*Chalcostigma ruficeps*	I, N	LC	This study
Trochilidae	*Chalcostigma stanleyi*	I, N	LC	9,10,12
Trochilidae	*Chlorostilbon aureoventris [lucidus]*	I, N	LC	5,10
Trochilidae	*Coeligena torquata*	I, N	LC	10
Trochilidae	*Coeligena violifer*	I, N	LC	10,12
Trochilidae	*Colibri coruscans*	I, N	LC	3,5,6,9,12,13
Trochilidae	*Colibri serrirostris*	I, N	LC	12,13
Trochilidae	*Colibri thalassinus*	I, N	LC	5
Trochilidae	*Ensifera ensifera*	I, N	LC	5,10
Trochilidae	*Eriocnemis glaucopoides*	I, N	LC	10
Trochilidae	*Heliangelus amethysticollis*	I, N	LC	10
Trochilidae	*Lesbia nuna*	I, N	LC	5,9,10
Trochilidae	*Metallura aenocauda [aeneocauda]*	I, N	LC	10
Trochilidae	*Metallura tyrianthina*	I, N	LC	3,5,9,10,12
Trochilidae	*Microstilbon burmeisteri*	I, N	LC	5
Trochilidae	*Oreotrochilus adela*	I, N	NT	3,5,9,10,13
Trochilidae	*Oreotrochilus estella*	I, N	LC	3,5,6,9,10,12
Trochilidae	*Oreotrochilus leucopleurus*	N	LC	13
Trochilidae	*Patagona gigas*	I, N	LC	3,5,6,9,10,13
Trochilidae	*Pterophanes cyanopterus*	I, N	LC	3,5,10,11
Trochilidae	*Sappho sparganura*	N	LC	3,5,6,9,10,13
Trochilidae	*Agleaectis pamela*	N	NR	3,5,10,12,13
Troglodytidae	*Cistothorus platensis*	I	LC	10

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Troglodytidae	*Troglodytes aedon*	I	LC	3,9,5,6,10,12,13
Troglodytidae	*Troglodytes solstitialis*	I	LC	10
Trogonidae	*Trogon personatus*	FG, I	LC	This study
Turdidae	*Catharus ustulatus*	FG	LC	5
Turdidae	*Turdus amaurochalinus*	I, FG	LC	5,10
Turdidae	*Turdus chiguanco*	I, FG	LC	3,5,6,9-14
Turdidae	*Turdus fuscater*	I, FG	LC	3,5,9,10,12
Turdidae	*Turdus serranus*	I	LC	12
Tyrannidae	*Agriornis andicola [albicauda]*	I, O	V, V	9-11
Tyrannidae	*Agriornis montana [montanus]*	I, O	LC	5,9,10
Tyrannidae	*Anairetes alpinus*	I	E, E	9,10,17
Tyrannidae	*Anairetes flavirostris*	I	LC	10,14
Tyrannidae	*Anairetes parulus*	I	LC	3,5,6,9-12,14,16
Tyrannidae	*Camptostoma obsoletum*	I	LC	5
Tyrannidae	*Casiornis rufa [rufus]*	I	LC	11
Tyrannidae	*Cnemarchus erythropygius*	I	LC, E	9
Tyrannidae	*Contopus fumigatus*	I	LC	This study
Tyrannidae	*Elaenia albiceps*	I	LC	3,5,6,10,12
Tyrannidae	*Elaenia pallatangae*	I	LC	10
Tyrannidae	*Elaenia strepera*	I, FG	LC	This study
Tyrannidae	*Hirundinea ferruginea*	I	LC	3,5,10
Tyrannidae	*Knipolegus aterrimus*	I	LC	3,5,10
Tyrannidae	*Mecocerculus hellmayri*	I	LC	11
Tyrannidae	*Mecocerculus leucophrys*	I	LC	3,5,6,9-14,16
Tyrannidae	*Mionectes striaticollis*	I, FG	LC	This study
Tyrannidae	*Muscisaxicola alpina [alpinus+griseus]*	I, FG	LC	This study
Tyrannidae	*Muscisaxicola cinerea [cinereus]*	I	LC	5
Tyrannidae	*Muscisaxicola flavinucha*	I	LC	This study
Tyrannidae	*Muscisaxicola frontalis*	I	LC	This study
Tyrannidae	*Muscisaxicola maculirostris*	I, FG	LC	5,10
Tyrannidae	*Muscisaxicola rufivertex*	I, FG	LC	10
Tyrannidae	*Myiarchus tuberculifer*	I	LC	5,10
Tyrannidae	*Myiodynastes chrysocephalus*	I	LC	10
Tyrannidae	*Myiophobus ochraceiventris*	I	LC	This study
Tyrannidae	*Myiotheretes fuscorufus*	I	LC	12
Tyrannidae	*Myiotheretes striaticollis*	I	LC	5
Tyrannidae	*Ochthoeca fumicolor*	I	LC	5,9,10
Tyrannidae	*Ochthoeca leucophrys*	I	LC	3,5,6,9-14,16
Tyrannidae	*Ochthoeca oenanthoides*	I	LC	3,5,6,9,10-14,16
Tyrannidae	*Ochthoeca rufipectoralis*	I	LC	3,5,9,10,12
Tyrannidae	*Ochthoeca spodionota*	I	NR	This study
Tyrannidae	*Phyllomyias nigricapillus [nigrocapillus]*	I	LC	10
Tyrannidae	*Phyllomyias uropygialis*	I	LC	This study
Tyrannidae	*Pitangus sulphuratus*	I	LC	5
Tyrannidae	*Polioxolmis rufipennis*	I	LC	3,5,9-11,13
Tyrannidae	*Satrapa icterophrys*	I	LC	5
Tyrannidae	*Serpophaga munda*	I	LC	3,5
Tyrannidae	*Stigmatura budytoides*	I	LC	This study
Tyrannidae	*Sublegatus modestus*	I	LC	5
Tyrannidae	*Suiriri suiriri*	I	LC	5,10
Tytonidae	*Tyto alba*	C	LC	5
Vireonidae	*Cyclarhis gujanensis*	I, FG	LC	5,10,11
Vireonidae	*Vireo olivaceus*	I	LC	This study

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Mammals				
Abrocomidae	*Abrocoma sp.*	H	NR	23
Camelidae	*Vicugna vicugna*	H	LC	25,26
Canidae	*Lycalopex culpaeus [Pseudalopex]*	C, O	LC	2,23,25,26
Caviidae	*Cavia tschudii*	H	LC	23
Caviidae	*Galea musteloides*	H	LC	23
Cervidae	*Hippocamelus antisensis*	H	V, E	23
Cervidae	*Mazama chunyi*	H	V, V	21
Chinchillidae	*Lagidium viscacia*	H	LC	23,25,26
Cricetidae	*Akodon aerosus*	I	LC	23
Cricetidae	*Akodon albiventer*	O, I	LC	23,25,26
Cricetidae	*Akodon boliviensis*	I, G	LC	2,23
Cricetidae	*Akodon fumeus*	I	LC	23
Cricetidae	*Akodon kofordi*	I	LC	23
Cricetidae	*Akodon lutescens*	I	LC	23
Cricetidae	*Akodon subfuscus*	I	LC	23,24
Cricetidae	*Andinomys edax*	H	LC	2,23
Cricetidae	*Bolomys amoenus [Necromys]*	NR	LC	23
Cricetidae	*Bolomys lactens [Necromys]*	NR	LC	2
Cricetidae	*Calomys lepidus*	NR	LC	23
Cricetidae	*Chinchillula sahamae*	H	LC	25,26
Cricetidae	*Chroeomys andinus [Abrothrix]*	I	LC	23
Cricetidae	*Chroeomys jelskii [Abrothrix]*	I, H	LC	23,25,26
Cricetidae	*Microryzomys minutus*	I	LC	23
Cricetidae	*Neotomys ebriosus*	H	LC	2
Cricetidae	*Oligoryzomys destructor*	I, H	LC	23
Cricetidae	*Oligoryzomys sp. B*	I, H	NR	23
Cricetidae	*Oxymycterus hiska*	I	LC, NT	23
Cricetidae	*Oxymycterus paramensis*	I	LC	23,25
Cricetidae	*Phyllotis cf. osilae A*	O	NR	23[f]
Cricetidae	*Phyllotis cf. osilae B*	O	NR	23[f]
Cricetidae	*Phyllotis cf. xanthopygus*	O, I	LC	23,25,26
Cricetidae	*Phyllotis osilae*	O	LC	2,23
Cricetidae	*Thomasomys n. sp.*	I	NR	23
Didelphidae	*Thylamys pallidior*	I	LC	17,23,25,26
Didelphidae	*Thylamys venustus*	I	DD	2
Felidae	*Oncifelis geoffroyi [Leopardus geoffroyi]*	C	NT, NT	2
Felidae	*Oncifelis pajeros [Lynchailurus]*	C	NR	2,25
Felidae	*Puma concolor*	C	LC	2,23,25,26
Mephitidae	*Conepatus chinga*	O	LC	2
Mustelidae	*Galictis cuja*	O	LC	2,22
Myrmecophagidae	*Tamandua tetradactyla*	I	LC	2
Phyllostomidae	*Sturnira erythromos*	F	LC	2
Ursidae	*Tremarctos ornatus*	O	V, V	4
Vespertilionidae	*Histiotus montanus*	I	LC	2
Vespertilionidae	*Lasirus cinereus*	I	NR	2
Vespertilionidae	*Myotis oxyotus*	I	LC	2
Reptiles				
Colubridae	*Oxyrhopus rhombifer*	C, I	NR	1
Colubridae	*Philodryas psammophidea*	C	NR	1
Colubridae	*Waglerophis merremi*	C	NR	1
Liolaemidae	*Liolaemus alticolor*	I	NR	1
Liolaemidae	*Liolaemus variegatus*	I	NR, V	1
Scincidae	*Mabuya cochabambae*	I	NR, V	1
Tropiduridae	*Stenocercus marmoratus*	I	NR	1
Viperidae	*Bothrops jonathani*	C	NR, V	1

Family	Species[a]	Trophic Niche[b]	IUCN Criteria[c,d]	References[e,g]
Lepidoptera				
Heliconiidae	*Dione glycera*	N	NR	20
Hesperiidae	*Hylephila isonira*	N	NR	20
Hesperiidae	*Hylephila peruana*	NR	NR	20
Hesperiidae	*Thespieus fassli*	NR	NR	20
Lycaenidae	*Itylos titicaca*	NR	NR	20
Lycaenidae	*Madeleinea lea*	N	NR	20
Lycaenidae	*Madeleinea moza*	N	NR	20
Lycaenidae	*Paralycaeides vapa*	N	NR	20
Lycaenidae	*Penaincisalia sp.*	NR	NR	20
Lycaenidae	*Rhamma brunea*	NR	NR	20
Lycaenidae	*Rhamma lapazensis*	N	NR	20
Nymphalidae	*Callicore sorana*	NR	NR	20
Nymphalidae	*Junonia vestina*	N	NR	20
Nymphalidae	*Marpesia chiron*	NR	NR	20
Nymphalidae	*Methona confusa*	NR	NR	20
Nymphalidae	*Vanessa altissima*	N	NR	20
Nymphalidae	*Vanessa braziliensis*	N	NR	20
Nymphalidae	*Vanessa carye*	N	NR	20
Nymphalidae	*Yramea inca*	NR	NR	20
Nymphalidae	*Yramea sobrina*	NR	NR	20
Papilionidae	*Pterourus scamander*	N	NR	20
Pieridae	*Anteus clorinde*	NR	NR	20
Pieridae	*Colias euxhante*	N	NR	20
Pieridae	*Eurema leuce*	NR	NR	20
Pieridae	*Phoebis neocypris*	NR	NR	20
Pieridae	*Phulia paranympha*	NR	NR	20
Pieridae	*Tatochila distincta*	NR	NR	20
Pieridae	*Tatochila mercedis*	N	NR	20
Pieridae	*Tatochila orthodice*	N	NR	20
Pieridae	*Tatochila sp.*	N	NR	20
Pieridae	*Tatochila stigmadice*	N	NR	20
Pieridae	*Teriocolias zelia*	N	NR	20
Pieridae	*Zerene cesonia*	N	NR	20
Satyridae	*Punargentus angusta*	NR	NR	20
Sphingidae	*Aellopos titan*	NR	NR	20

[a] The species names follows the authors. The names in brackets are synonyms used for the species in the IUCN red list or other names used by authors.

[b] N= Nectarivorous, F= frugivorous, FG= frugigranivorous, G= granivorous, I= insectivorous, C= carnivorous, O= omnivorous, B= browser, H= herbivorous, NR= not reported.

[c] LC= Least concern, NT=near threatened, V= vulnerable, E= endangered, NR= not reported.

[d] In case of two values, before the comma are the IUCN categories according to (15) and after the comma are the IUCN categories according to (18).

[e] 'This study' means that this data is from JF's databases.

[f] Both species of *Phyllotis cf. osilae* (A and B) are similar to *P. osilae* but do not coincide with important characteristics and may represent no described species or subspecies (21).

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Appendix 3. Localities and areas matching our criteria for analyzing the relationship between species diversity and climate. For references see text.

Localities or areas	Latitude (S)	Longitud (W)	Origen of coordinates[a]	Elevation range (m)	Reference
Plants					
Sehuencas-Monte Punco	17°30'-35'	65°15'-20'	Mercado-Ustariz (1998)	3220-3700	Mercado-Ustariz (1998)
Mojón	17°29'	65°25'	Fernández-Terrazas & Stahl (2002)	2950-3340	Fernández-Terrazas (1997)
Lope Mendoza	17°30'	65°22'	Fernández-Terrazas & Stahl (2002)	3110-3460	Fernández-Terrazas (1997)
PNT km12	17°19'	66°08'	Fernández-Terrazas & Stahl (2002)	3485-3670	Fernández-Terrazas (1997)
Km 10 Liriuni	17°17'	66°17'	Fernández-Terrazas & Stahl (2002)	3600-3750	Fernández-Terrazas (1997)
Taquiña	17°18'	66°12'	Fernández-Terrazas & Stahl (2002)	3715-4040	Fernández-Terrazas (1997)
Frente Laphia	17°18'	66°13'	Fernández-Terrazas & Stahl (2002)	3250-3430	Fernández-Terrazas (1997)
Kewiñal	17°33'	65°42'	Fernández-Terrazas & Stahl (2002)	3250-3500	Fernández-Terrazas (1997)
Zapata Rancho	17°27'	65°40'	Fernández-Terrazas & Stahl (2002)	3200-3210	Fernández-Terrazas (1997)
Sacha Loma	17°44'	65°34'	Herzog *et al.* (2003)	3700-3800	Fernández *et al.* (2001)
PNT km 12	17°19'	66°08'	Balderrama (2006)	3350-3900	Balderrama & Ramirez (2001)
San Miguel	17°16'	66°20'	Balderrama (2006)	3600-4000	Balderrama & Ramirez (2001)
Challapata-Potosí before Yocalla	19°23'	65°55'	Geody	4080	Navarro *et al.* (2005)
Potosí-Camargo km20	19°47'	65°45'	Google Earth	3900	Navarro *et al.* (2005)
Potosí-Uyuni km15	19°40'	65°49'	Google Earth	3920	Navarro *et al.* (2005)
Potosí-Camargo past Padcoyo	20°24'	65°18'	Geody	3400-3470	Navarro *et al.* (2005)
Cerro Azanaques	18°56'	66°40'	Geody	3730	Navarro *et al.* (2005)
Tarabuco	19°10'	64°55'	Geody	3200	Navarro *et al.* (2005)
Curahuara-Sajama km22	17°52'	68°35'	Geody + Google Earth	3980	Navarro *et al.* (2005)
Berenguela-La Paz km5	17°17'	69°11'	Google Earth	3950	Navarro *et al.* (2005)
Sajama	18°08'	68°49'	Google Earth	4380-4750	Navarro *et al.* (2005)
Zapata Rancho	17°27'	65°40'	Fernández-Terrazas & Stahl (2002)	3200-3210	Navarro *et al.* (2005)
Bolivar	17°58'	66°32'	Google Earth	3940-4020	Navarro *et al.* (2005)
Kewiñal	17°33'	65°42'	Fernández-Terrazas & Stahl (2002)	3250-3500	Navarro *et al.* (2005)
Mojon	17°29'	65°25'	Fernández-Terrazas & Stahl (2002)	2950-3340	Navarro *et al.* (2005)
Pirhua-Pirhua	18°19'	64°59'	Geody + Google Earth	3000-3030	Navarro *et al.* (2005)
Cueva over Liriuni	17°17'	66°17'	Google Earth	3420-3600	Navarro *et al.* (2005)
Liriuni	17°17'	66°17'	Fernández-Terrazas & Stahl (2002)	3600-3750	Navarro *et al.* (2005)
Laphia	17°18'	66°13'	Fernández-Terrazas & Stahl (2002)	3420-3450	Navarro *et al.* (2005)
PNT	17°19'	66°08'	Fernández-Terrazas & Stahl (2002)	3485-3670	Navarro *et al.* (2005)
Taquiña	17°18'	66°12'	Fernández-Terrazas & Stahl (2002)	3715-4040	Navarro *et al.* (2005)

Localities or areas	Latitude (S)	Longitud (W)	Origen of coordinates[a]	Elevation range (m)	Reference
Birds					
Sacha Loma	17°44'	65°34'	Herzog *et al.* (2003)	3710-3880	Herzog *et al.* (2003)
PNT12	17°19'	66°08'	Balderrama (2006)	3350-3650	Balderrama & Ramirez (2001)
San Miguel	17°16'	66°20'	Balderrama (2006)	3700-3900	Balderrama & Ramirez (2001)
Taquiña	17°18'	66°10'	Balderrama (2006)	2800-3800	Balderrama (2006)
Candelaria N	17°15'	65°55'	Balderrama (2006)	3550-3850	Balderrama (2006)
Candelaria S	17°16'	65°54'	Balderrama (2006)	3300-3600	Balderrama (2006)
PNT 12	17°19'	66°08'	Balderrama (2006)	3350-3650	Balderrama (2006)
Thola Pujru	17°17'	66°13'	Balderrama (2006)	3400-3600	Balderrama (2006)
San Miguel	17°16'	66°20'	Balderrama (2006)	3700-3900	Balderrama (2006)
Laphia	17°15'	66°14'	Balderrama (2006)	3600-3900	Balderrama (2006)
Chorojo	17°27'	66°28'	Balderrama (2006)	3700-3800	Balderrama (2006)
Pintumayu	17°19'	66°09'	Balderrama (2006)	3600-3900	Balderrama (2006)
Tiraque	17°24'	65°42'	This study	3440	Fjeldså & Kessler (1996) Fj&K178
Khenwa Sandora	17°41'	66°30'	This study	3900	Fjeldså & Kessler (1996) Fj87_74
Tunari	17°18'	66°09'	This study	3960	Fjeldså & Kessler (1996) Fj87_77
Kehuiñal	17°33'	65°43'	This study	3440	Fjeldså & Kessler (1996) Fj87_80
Llallagua	16°50'	67°56'	This study	4100	Fjeldså & Kessler (1996) FJ87_91
Sajama	18°10'	68°57'	This study	4300	Fjeldså & Kessler (1996) Fj87-94a
Sajama	18°10'	68°53'	This study	4600	Fjeldså & Kessler (1996) Fj87_94c
W Sajama	18°04'	68°04'	This study	4370	Fjeldså & Kessler (1996) Fj87_94d
Portillo	20°42'	64°42'	This study	3000	Fjeldså & Kessler (1996) Fj&M1
W Azurduy	20°06'	64°25'	This study	2600	Fjeldså & Kessler (1996) Fj&M9
Honduras	21°59'	64°36'	This study	2700	Fjeldså & Kessler (1996) Fj&M10
La Cumbre	22°00'	64°36'	This study	3030	Fjeldså & Kessler (1996) Fj&M11
Rio Escalera	22°00'	64°34'	This study	2800	Fjeldså & Kessler (1996) Fj&M12
Alto de Minas	21°58'	64°36'	This study	3300	Fjeldså & Kessler (1996) Fj&M14
Acasio	18°02'	66°04'	This study	3500	Fjeldså & Kessler (1996) DK95-1
Canto Monte	17°30'	65°26'	This study	3000	Fjeldså & Kessler (1996) DK95-2
Lajamayo	18°25'	65°17'	This study	2750	Fjeldså & Kessler (1996) DK95-3
Tarabuco	19°10'	64°57'	This study	3300	Fjeldså & Kessler (1996) DK95-4
La Candelaria	17°15'	65°52'	This study	3750	Andersen *et al.* (1999) CA3
Kehuiñal	17°33'	65°43'	This study	3500	Andersen *et al.* (1999) CK1

Localities or areas	Latitude (S)	Longitud (W)	Origen of coordinates[a]	Elevation range (m)	Reference
Cuchillani	17°20'	66°06'	This study	3780	Andersen *et al.* (1999) CU3
Khalani	17°45'	66°30'	This study	3600	Andersen *et al.* (1999) KH2+3
Kehuiña Pampa	17°29'	66°23'	This study	3050	Andersen *et al.* (1999) KP1-3
Liriuni	17°17'	66°20'	This study	3700	Andersen *et al.* (1999) LI3
Lopez Mendoza	17°30'	65°22'	This study	3600	Andersen *et al.* (1999) LM1-3
Mojon	17°32'	65°21'	This study	3200	Andersen *et al.* (1999) MO1-4
Oropeza	17°38'	65°30'	This study	3500	Andersen *et al.* (1999) OR1-2
San Miguel	17°16'	66°20'	This study	3900	Andersen *et al.* (1999) SM1-5
Tiquipaya	17°17'	66°14'	This study	3800	Andersen *et al.* (1999) TI1-5
Tunari	17°19'	66°09'	This study	4000	Andersen *et al.* (1999) TU 4-5
Casayvinto	16°51'	66°38'	This study	3350	This study
Cocapata	16°47'	66°41'	This study	3215	This study
Cocapata	16°48'	66°41'	This study	3350	This study
Sailapata	16°55'	66°51'	This study	3050	This study
Jatun Potrero	17°32'	65°12'	This study	3100	This study
Jatun Potrero	17°32'	65°11'	This study	3300	This study
Siete Quebradas	17°29'	65°12'	This study	3150	This study
Jatun Potrero	17°30'	65°11'	This study	3600	This study
W Tarabuco	19°09'	64°58'	This study	3400	This study
Mammals (areas)					
Ulla Ulla	14°49'-15°06'	68°55'-69°04'	Yensen & Tarifa (2002)	3800	Yensen & Tarifa (2002)
Tacacoma	15°36'-37'	68°39'-41'	Yensen & Tarifa (2002)	3800	Yensen & Tarifa (2002)
Mayca Mayu	17°05'	66°05'-06'	Yensen & Tarifa (2002)	3300-3400	Yensen & Tarifa (2002)
Carrasco	17°30'-34'	65°17.5'-25'	Yensen & Tarifa (2002)	3200-3700	Yensen & Tarifa (2002)
Kheñwa Sandora	17°40'-45'	66°27'-29'	Yensen & Tarifa (2002)	3700	Yensen & Tarifa (2002)
Sajama	18°02'-11'	68°55'-57'	Yensen & Tarifa (2002)	4240-4600	Yensen & Tarifa (2002)
Acacio	18°06'	66°13'	Yensen & Tarifa (2002)	3500-3700	Yensen & Tarifa (2002)
Icla	19°34'	64°39'-40'	Yensen & Tarifa (2002)	3700-3800	Yensen & Tarifa (2002)
Wara Wara	19°49'	65°08'	Yensen & Tarifa (2002)	3700	Yensen & Tarifa (2002)
Camargo	20°19'-26'	65°07'-08'	Yensen & Tarifa (2002)	3200-3600	Yensen & Tarifa (2002)
Sama	21°42'	65°09'	Yensen & Tarifa (2002)	4000	Yensen & Tarifa (2002)

[a] Geody= http://www.geody.com, Geody+Google Earth: Coordinates obtained from Geody and elevation from Google Earth.

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