

Fragment size, pollination efficiency and reproductive success in natural populations of wind-pollinated *Polylepis australis* (Rosaceae) trees

Peggy Seltmann^{a,*}, Daniel Renison^b, Andrea Cocucci^c, Isabell Hensen^a, Klaus Jung^d

^a*Institute of Geobotany and Botanical Garden, Martin-Luther-University Halle-Wittenberg, Am Kirchtor 1, 06108 Halle/Saale, Germany*

^b*Cátedra de Ecología General, National University of Córdoba, Av. V. Sarsfield 299, 5000 Córdoba, Argentina*

^c*Instituto Multidisciplinario de Biología Vegetal (IMBiV), National University of Córdoba, Av. V. Sarsfield 299, 5000 Córdoba, Argentina*

^d*Department of Chemical Ecotoxicology, Centre for Environmental Research Leipzig-Halle, Permoserstraße 15, 04318 Leipzig, Germany*

Received 24 July 2006; accepted 2 December 2006

Abstract

It is well known that habitat fragmentation is likely to negatively affect the reproductive success of a species. Despite this fact, studies on the effects of fragmentation on reproductive success in combination with effects on natural pollination of wind-pollinated tree species are very rare. In this study, we analyzed the relationships between fragment size, components of pollination efficiency, reproductive success and progeny vigour of the highly fragmented populations of the wind-pollinated treeline species *Polylepis australis* (Rosaceae) in Argentina. We conducted our study in the high mountains of Córdoba, comparing pollination efficiency and reproduction between four fragment sizes. Most of our results indicate that *P. australis* is currently insensitive to fragmentation. However, our study revealed also a linear increase in leaf area and biomass of 40-day-old seedlings with increasing fragment size. Inbreeding problems through fragmentation as well as decreased habitat quality in small fragments are discussed as possible causes for the impaired progeny vigour.

© 2007 Elsevier GmbH. All rights reserved.

Keywords: Fragmentation; Natural pollination; Reproductive success; Wind pollination; Fragment size

Introduction

Forest ecosystems have been and continue to be destroyed rapidly by human activities, prompting a need for information on the reproductive responses of tree species to fragmentation. Small and isolated forest fragments experience a reduction in the number of

reproductive individuals, representing a decrease in the number of pollen donors and the quantity of pollen deposited (e.g. Aguilar and Galetto, 2004; Aizen and Feinsinger, 1994; Cunningham, 2000; Sih and Baltus, 1987). Furthermore, smaller forest fragments also show greater sensitivity to environmental variation and demographic stochasticity (Heschel and Paige, 1995; Menges, 1991). Thus, fragmentation may negatively affect reproductive success of a species by reducing pollen deposition and pollen quality (e.g. Aizen and Feinsinger, 1994; Cascante et al., 2002; Cunningham,

*Corresponding author. Tel./fax: + 59 345 5526261.

E-mail address: peggy.seltmann@botanik.uni-halle.de (P. Seltmann).

2000; Ghazoul et al., 1998), negatively affecting fruit and seed production (e.g. Aguilar and Galetto, 2004; Aizen and Feinsinger, 1994; Cascante et al., 2002) and reducing progeny vigour (e.g. Cascante et al., 2002).

Because the major effects of fragmentation on forests may directly or indirectly result from changes in forest size, studies utilizing extant populations of different sizes can provide insight into some of the potential consequences of habitat fragmentation (Hendrix and Kyhl, 2000). Numerous studies on effects of plant population size have shown reproductive difficulties in small populations due to genetic problems or/and degradation of habitat quality within fragments (e.g. Aizen and Feinsinger, 1994; Harrison and Bruna, 1999; Heschel and Paige, 1995; Menges, 1991; van Treuren et al., 1991). However, such studies have been rare for wind-pollinated tree species and findings have been mixed. For instance, whereas fragmentation has generally been found to have a negative effect on progeny vigour, evidence also exists indicating that fragmentation can be associated with enhanced pollen dispersion and reproductive success of remaining trees via reduced competition and enhanced wind dispersal (Smouse and Sork, 2004).

Here we study the relationships between fragment size and reproduction in fragments of *Polylepis australis*, a wind-pollinated treeline species endemic to Argentina. Specifically, we examined the association between *P. australis* fragment size and the following parameters: (1) number of pollen grains on the stigmas, germination of pollen grains and number of pollen tubes found at the top of the styles after natural pollination; (2) seed germination; and (3) progeny vigour.

Materials and methods

Study species and area

The wind-pollinated trees and shrubs of the genus *Polylepis* R. & P. (Rosaceae, Sanguisorbeae) constitute the potential natural vegetation of the South American high mountains, but are today mostly restricted to relictual populations due to anthropogenic deforestation (Ellenberg, 1958; Kessler, 1995; Schmidt-Lebuhn et al., 2006). Ecologically, the genus is especially noted for reaching the highest elevations among woody plants in the Neotropics, if not worldwide (Jordan, 1983; Purcell et al., 2004).

P. australis is endemic to the Central and North-western mountains of Argentina (Simpson, 1979). Its annually produced racemiform inflorescences are 1.8–7.3 cm long catkins that may carry up to 12%, wind-pollinated flowers with red, conspicuous anthers uniformly expanded and fimbriate stigmas (one per

flower, Simpson, 1986). Autogamy is precluded by protogyny. Seltmann et al. (accepted a, submitted b) reported reduced reproductive success in *P. australis* resulting from artificial selfing and from mating with near neighbours. Furthermore, these studies found indication for gametophytic self-incompatibility mechanisms preventing self-fertilization. Flowers generally develop one ovule (with a few exceptions where two are formed) and fruits are mostly single-seeded nutlets (hereafter referred to as seeds) enclosed in a turbinate and winged receptaculum.

We conducted this study in the upper portion of the Córdoba mountains (31°34'S, 64°50'W, 1800–2300 m a.s.l.). Average annual rainfall is 840 mm, and mean temperature of the coldest and warmest months at 2100 m a.s.l. are 5.0 and 11.4 °C (Cabido et al., 1987). As a consequence of three centuries of domestic grazing and anthropogenic fires, the present-day vegetation consists of a mosaic of tussock grasslands, grazing pasture, granite outcrops, eroded areas with exposed rock surfaces, and fragments of *P. australis* forests whose density and size is mainly determined by past human disturbances interacting with natural topographic features (Cingolani et al., 2004; Renison et al., 2006).

Selection of trees

To examine the association between fragment size, natural pollination, seed germination and progeny vigour, we compared trees that were distributed in 12 fragments which we classified into four fragment sizes (three fragments each). The distance between individual fragments was at least 4 km. Fragment sizes (FS) were defined by the number of trees in the fragment: FS 1: 1–4 trees; FS 2: 8–20 trees; FS 3: 100 to ~500 trees; FS 4: > 1000 trees. All trees selected were reproductive adults with similar height (2.5–3 m). Each focal tree was separated by at least 20 m from one another within each fragment. We always tried to distribute sampling trees as evenly as possible between the 12 fragments, but sample sizes varied due to flower and seed availability and later differential progeny production.

Natural pollination

In order to determine the association between fragment size and pollination efficiency, we sampled a total of 39 trees: 6–13 trees per fragment size distributed within the 12 fragments. Per tree, we collected 5–10 styles from senescing flowers of different inflorescences each during the flowering peak of 2003; resulting in a total of 342 styles. Flower styles were fixed in 70% ethanol, softened in KOH for 1 h, and stained (Martin, 1959). The number of pollen grains on the stigma, the number of germinated pollen grains on the stigma and

the number of pollen tubes at the top of the styles were counted using the aniline blue technique and epifluorescence microscopy (Kearns and Inouye, 1993; Martin, 1959). We did not count pollen tubes at the base of the styles because tubes within the styles were not distinguishable.

Seed germination and seedling vigour

In order to determine the association between fragment size, and seed germination and seedling vigour, we sampled a total of 54 trees: 10–16 trees per fragment size distributed within the 12 fragments. Trees used for the study on natural pollination were included in this sample and we collected a sample of 20 fruits per tree, giving a total of 1080 seeds. Germination of seeds was tested in petri dishes at a temperature of 20 °C/10 °C (12 h of light/12 h of darkness) in a climate chamber with a warm white light source. The experiments were terminated after 45 days, when germination was zero. The germination percentage was calculated as the ratio of number of seeds germinated to the total number of seeds used for each tree.

Seedlings from 46 trees representing the 12 fragments of the four fragment sizes were then randomly selected and transplanted to individual flower pots of 10 cm diameter immediately after germination. Transplanted seedlings were then cultivated in the greenhouse at 25–28 °C and watered equally every 3–4 days (FS 1: seedlings from 6 trees, FS 2: seedlings from 12 trees, FS 3 and FS 4: seedlings from 14 trees each; 1–9 seedlings per tree; totalling 165 seedlings). After 40 days of seedling growth, the following variables of seedling vigour were obtained: (1) number of leaves; (2) total leaf area, using a Delta-T Image Analysis System; (3) seedling dry biomass; and (4) N metabolism capacity. We included the N metabolism capacity as a variable of seedling vigour because results of Seltmann et al. (submitted b) indicated significant biparental inbreeding depression resulting from crosses with nearby neighbours and with individuals of nearby fragments, evident in decreased nitrogen metabolism capacity of the seedlings. In order to specify the N metabolism capacity (measured as their ^{15}N uptake), we applied the ^{15}N tracer technique using a ^{15}N -labeled tracer to detect the destiny of N-atoms (e.g. Arndt et al., 1998; Jung et al., 1999; Schulz et al., 2001; Seltmann et al., submitted b; Sutter et al., 2002). Seedling roots were carefully washed in distilled water and incubated in test tubes in standard media with constant stable isotope abundance (prepared by dissolving 2 g K^{15}NO_3 with 95 atm% ^{15}N in 1 L distilled water) for 6 h. Thereafter, the plant material was dried for 48 h in silica gel until plant samples were completely dry. Following weight measurement of the dried plant material (to detect seedling biomass as

mentioned above), seedling roots were sectioned. Subsequently, plant material was pulverized in a ball mill and subsamples of about 1.5 mg were weighed into ultra-clean tin capsules (two capsules per seedling). The amount of ^{15}N that was incorporated by the seedling was determined by isotope ratio mass spectroscopy (IRMS) using an elemental analyzer in connection with a gas isotope mass spectrometer (IRMS-Delta Plus XP; ThermoFinnigan, Bremen, Germany).

Statistical analysis

The following natural pollination response variables (serving as components of pollination efficiency) were analyzed: (1) number of pollen grains per stigma. As Stephenson et al. (1995) mentioned that the number of pollen grains in open-pollinated pistils usually has to exceed the number of ovules for fruit set to occur, and ovaries of *P. australis* generally develop only one ovule per ovary; we also included (2) percentage of flowers with two or more pollen grains on the stigma; (3) percentage of flowers with two or more germinated pollen grains on the stigma; (4) percentage of flowers with two or more pollen tubes at the top of the style. Our response variables relating to seed germination and seedling vigour at 40 days were; (5) percentage of germinated seeds per tree; (6) number of leaves; (7) total seedling leaf area; (8) seedling dry biomass; and (9) seedling N metabolism capacity.

We tested whether there was an effect of fragment size on our response variables conducting a GLM ANOVA (Nested ANOVA) for each of our response variables. ‘Fragment size’ was the fixed main factor, ‘fragment’ a random nested factor within ‘fragment size’, and ‘maternal tree’ a random nested factor within ‘fragment’. We used Student–Newman–Keuls post-hoc test to evaluate which fragment sizes differed in the response variables. We estimated the power of the GLM ANOVA’s for each dataset, using a significance level of 0.05. In order to better approximate normality and homoscedasticity of residuals, percentages were arcsin squareroot transformed and data on stigma pollen load and seedling vigour were ln transformed prior to further analysis. All analyses were conducted using NCSS 2001 software.

Results

Natural pollination

The number of pollen grains per stigma ranged from 0 to 74 (mean 8, median 5). Flowers from the different fragment sizes received similar pollen loads on their stigmas (Fig. 1). Eighty-eight percent of all stigmas

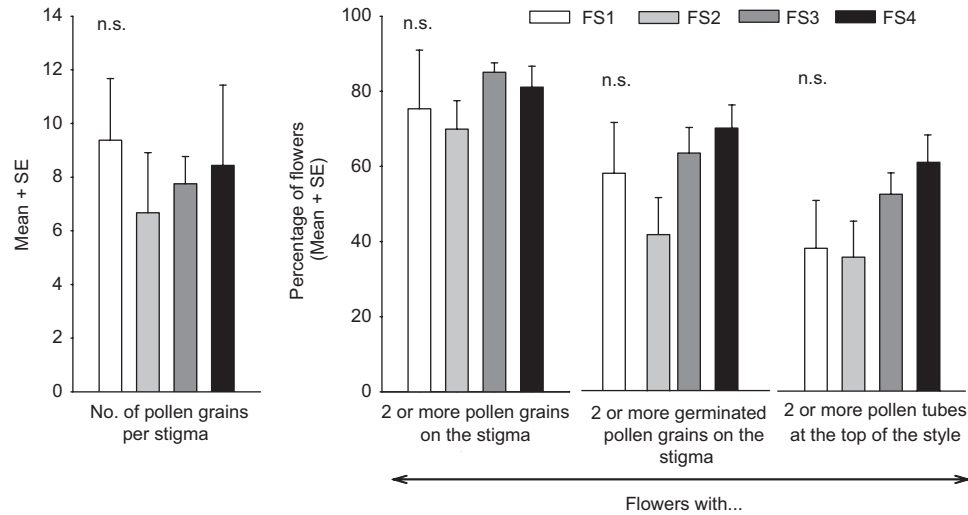


Fig. 1. Stigma pollen loads; and percentage of flowers with two or more pollen grains on the stigma, two or more germinated pollen grains on the stigma, and two or more pollen tubes at the top of the style for *Polylepis australis* trees of fragments belonging to different fragment sizes (FS): FS 1: 1–4 trees; FS 2: 8–20 trees; FS 3: 100 to ~500 trees; FS 4: > 1000 trees. n.s. = no significant differences between the four fragment sizes.

received at least one pollen grain and 78% received at least two pollen grains. Results indicated that not all pollen grains reaching the stigma were successful as 58% of the stigmas had at least two germinated pollen grains and only 47% had two or more pollen tubes at the top of the styles. None of the components of pollination efficiency was significantly related to fragment size (Table 1, Fig. 1) but there was a tendency of increasing percentages of germinated pollen grains on the stigma and of percentages of pollen tubes reaching the styles in trees derived from larger fragment sizes (Fig. 1). The power to detect an effect of the fragment size ranged from 9% to 29% (Table 1).

The effect of 'fragment' within 'fragment size' was significant for the variable "percentage of two or more pollen tubes at the top of the styles". In addition, the effect of maternal tree on "stigma pollen load" was significant while there was no significant maternal tree effect on any of the remaining components of pollination efficiency.

Seed germination and seedling vigour

Seed germination of all trees was highly variable and ranged from 0% to 75% with a median of 30%. The values representing the parameters of progeny vigour were rather variable with respect to leaf area and seedling biomass (mean leaf area: 998 mm², SD 511.9; mean seedling biomass: 36 mg, SD 17.5). The mean number of leaves was 4.5 (SD 0.8) and the mean ¹⁵N abundance in the seedlings reached 0.6 atm% (SD 0.1).

Nested ANOVA showed that germination of seeds obtained from the different trees was not significantly

affected by fragment size (Table 1, Fig. 2). However, leaf area as well as seedling biomass were significantly affected by fragment size (Table 1), whereby 40-day-old seedlings resulting from mother trees of the largest fragment size (FG 4) had a significantly greater leaf area and biomass than seedlings from the smallest fragment size (Fig. 2). Furthermore, the ¹⁵N uptake of the seedlings was significantly affected by fragment size (Table 1), although a Student–Newman–Keuls post-hoc test was unable to detect which were the fragment sizes that differed (Fig. 2). The number of leaves that had been developed after 40 days did not differ significantly between fragment sizes (Table 1, Fig. 2). The power to detect an effect of fragment size on seed germination and all four measurements of seedling vigour ranged from 32% to 74% (Table 1).

The effect of 'fragment' within 'fragment size' was significant for the leaf area and the biomass of the seedlings. In addition, the effect of maternal tree on the seedlings ¹⁵N uptake was significant whereas there was no significant maternal tree effect on any of the remaining parameters of seedling vigour (Table 1).

Discussion

Despite a large body of theory, few studies have directly assessed the association between fragment size, pollination and reproductive success in natural populations of tree species, and available data on insect-pollinated trees (e.g. Aguilar and Galetto, 2004; Aizen and Feinsinger, 1994; Cascante et al., 2002) have delivered rather contradictory results. Moreover, to

Table 1. Results of a Nested ANOVA with ‘fragment’ nested in ‘fragment size’ and ‘tree’ nested in ‘fragment’, and the corresponding power analysis for natural pollination, seed germination and parameters of seedling vigour of *Polylepis australis* trees from fragments belonging to different fragment sizes (* $p \leq 0.05$, *** $p < 0.001$)

Response variable	Source of variation	df	<i>F</i>	<i>p</i>	Power (Alpha = 0.05)
<i>Natural pollination</i>					
Stigma pollen load	Fragment size (A)	3	1.67	0.49	0.17
	Fragment B (A)	8	1.10	0.35	
	Tree C (AB)	27	3.24	0.00***	
Flowers with two or more pollen grains on the stigma	Fragment size (A)	3	0.30	0.83	0.09
	Fragment B (A)	8	1.68	0.15	
	Tree C (AB)	27			
Flowers with two or more germinated pollen grains on the stigma	Fragment size (A)	3	0.89	0.49	0.17
	Fragment B (A)	8	1.92	0.10	
	Tree C (AB)	27			
Flowers with two or more pollen tubes at the top of the style	Fragment size (A)	3	1.04	0.43	0.19
	Fragment B (A)	8	2.29	0.05*	
	Tree C (AB)	27			
Seed germination	Fragment size (A)	3	2.47	0.14	0.41
	Fragment B (A)	8	1.16	0.35	
	Tree C (AB)	42			
<i>Seedling vigour</i>					
No. of leaves	Fragment size (A)	3	1.90	0.21	0.32
	Fragment B (A)	8	0.81	0.60	
	Tree C (AB)	34	1.15	0.28	
Leaf area	Fragment size (A)	3	3.94	0.05*	0.61
	Fragment B (A)	8	3.23	0.01*	
	Tree C (AB)	34	0.76	0.82	
Seedling biomass	Fragment size (A)	3	4.16	0.05*	0.63
	Fragment B (A)	8	2.30	0.04*	
	Tree C (AB)	34	0.86	0.69	
N metabolism capacity (¹⁵ N uptake)	Fragment size (A)	3	5.17	0.03*	0.74
	Fragment B (A)	8	0.47	0.87	
	Tree C (AB)	34	1.77	0.01*	

Replications within trees are given for ‘stigma pollen load’ by the number of counted stigmas, and for all parameters of seedling vigour by the number of seedlings obtained from each mother tree.

our knowledge, no studies exist which have directly assessed the effect of fragment size on the natural pollination of wind-pollinated trees.

Our results indicate that there were differences between fragment sizes in variables related to reproductive success: an increasing progeny leaf area and biomass with increasing fragment size. In coincidence, during an ongoing reforestation project, Renison et al. (2005) found that seedling growth during their first 5 years was faster when seedlings were produced from seeds collected in a large forest than when collected in smaller fragments. In addition, our results revealed that seed germination was higher in the two larger fragment sizes than in the smaller ones, but this tendency did not result in significant differences.

The significant differences in progeny leaf area and biomass might be a consequence of a greater incidence of correlated paternity through increased mating between neighbour trees leading to inbreeding depression in small fragments. Small population size and the consequent relatively small number of distant pollen donors may increase the probability of mating with near neighbours (e.g. Bhausahab et al., 2005; Cascante et al., 2002; Quesada et al., 2001; Stephenson et al., 1995). However, this assumption is in contrast to the fact that the N metabolism capacity of seedlings did not increase with increasing fragment size. As mentioned above, Seltmann et al. (submitted b) showed decreased nitrogen metabolism capacity of seedlings that resulted from manual crosses with neighbouring trees.

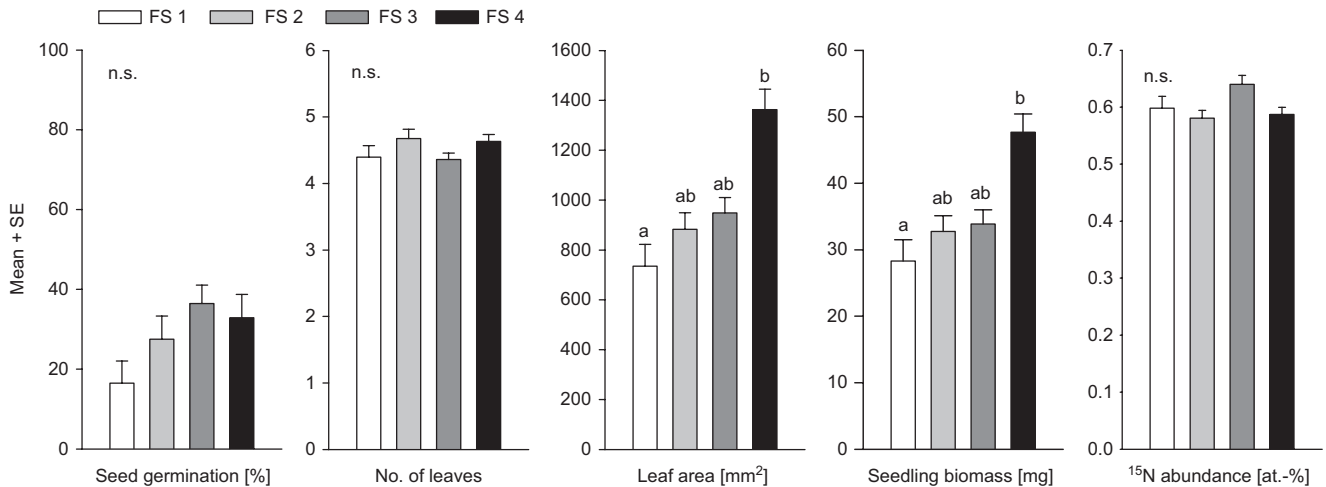


Fig. 2. Germination of seeds, and four vigour parameters of 40-day-old seedlings of *Polylophus australis* (leaf number, leaf area, seedling biomass, N metabolism capacity as measured by the ^{15}N uptake) produced by trees of fragments belonging to different fragment sizes (FS): FS 1: 1–4 trees; FS 2: 8–20 trees; FS 3: 100 to ~500 trees; FS 4: > 1000 trees. Different letters indicate significant differences; n.s. = no significant differences between the four fragment sizes.

A possible complementary explanation for the differences in leaf area and seedling biomass between fragments of different size is related to habitat quality. Empirical studies tend to suggest that fragmentation is more a matter of degradation of habitat (e.g. soil erosion, decreased water availability) within smaller fragments (Harrison and Bruna, 1999). As the environment in which the mother plant grows may influence its ability to provision seeds, the growing conditions of the parent plant influence both the number and quality of its progeny (e.g. Delph et al., 1997; Dolan, 1984; Gionali and González-Teuber, 2005; Weiner et al., 1997). The fact that we found differences in leaf area and biomass of the seedlings, but not their N uptake may suggest that *P. australis* seedling growth depends more on seed reserves and parental habitat quality than on the seedlings N metabolism capacity. In the case of *P. australis*, it is especially true that small fragments are in habitats where fires and livestock can eradicate large forests more easily (Renison et al., 2006; Teich et al., 2005). In accordance, Renison et al. (2005) found that anthropogenic soil degradation as a consequence of livestock pressure and fire utilization negatively affects seed quality in *P. australis*.

None of the differences in the components of pollination efficiency was significant. This, in combination with the results of the seedlings N metabolism capacity would indicate that *P. australis* pollination is currently insensitive to fragmentation. In accordance, Seltmann et al. (accepted a, submitted b) assumed reproductive and genetic connectivity of *P. australis* woodland fragments through effective pollen flow. However, the apparent tendency of increasing percentages of germinated pollen grains on the stigma and of

percentages of pollen tubes reaching the styles in trees derived from large fragment sizes (Fig. 1) could indicate the beginning of a decline in the naturally deposited outcrossed pollen load in small *P. australis* woodland fragments.

Furthermore, the fact that there was a decrease of 18% from the mean percentage of two or more pollen tubes at the top of the style to mean seed germination implies that there is a loss of viable *P. australis* seeds in each stage of the flower–seed forming process, possibly through arrest of pollen tube growth in the style, failures during fertilization, and/or embryo abortion.

Nonetheless, when one considers the results of decreased seedling leaf area and biomass in small fragments, it becomes apparent that, even if there is genetic connectivity between isolated fragments, this connectivity cannot compensate the loss of quality of the progeny produced by trees in small fragments and its negative effects on the regeneration of *P. australis* woodlands. Therefore, to maintain seedling vigour a clear conservation strategy is needed to avoid further fragmentation accompanied by ongoing degradation of *P. australis* habitat.

Acknowledgements

We are thankful to David Smaldone and Irene Lett for her help in the field, to Helga Schroeter and Ingrid Fluegel for technical support, and to Joe King for the revision of the manuscript. We would also like to thank the Volkswagen foundation for supporting the project and the National Parks Administration (Argentina) for providing working permits.

References

- Aguilar, R., Galetto, L., 2004. Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae). *Oecologia* 138, 513–520.
- Aizen, M.A., Feinsinger, P., 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco Dry Forest, Argentina. *Ecology* 75, 330–351.
- Arndt, K., Hofmann, D., Gehre, M., Krumbiegel, P., 1998. ¹⁵N investigation into the effect of a pollutant on the nitrogen metabolism of *Tetrahymena pyriformis* as a model for environmental medical research. *Env. Health Persp.* 106, 493–479.
- Bhauasheb, T., Rajanikanth, G., Ravikanth, G., Uma Shaanker, R., Ganeshiah, K.N., Kushalappa, C.G., 2005. Seedling mortality in two vulnerable tree species in the sacred groves of Western Ghats, South India. *Curr. Sci.* 88, 350–352.
- Cabido, M., Breimer, R., Vega, G., 1987. Plant communities and associated soil types in a high plateau of the Córdoba mountains, central Argentina. *Mountain Res. Dev.* 7, 25–42.
- Cascante, A., Quesada, M., Lobo, J.L., Fuchs, E.A., 2002. Effect of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conserv. Biol.* 16, 137–147.
- Cingolani, A.M., Renison, D., Zak, M.R., Cabido, M.R., 2004. Mapping vegetation in a heterogeneous mountain rangeland using Landsat data. An alternative method to define and classify land-cover units. *Remote Sens. Environ.* 92, 84–97.
- Cunningham, S.A., 2000. Depressed pollination in habitat fragments causes low fruit set. *Proc. R. Soc. London* 267, 1149–1152.
- Delph, L.F., Johannsson, M.H., Stephenson, A.G., 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* 78, 1632–1639.
- Dolan, R.W., 1984. The effect of seed size and maternal source on individual size in a population of *Ludwigia leptocarpa* (Onagraceae). *Am. J. Bot.* 71, 1302–1307.
- Ellenberg, H., 1958. Wald oder Steppe? Die natürliche Pflanzendecke der Anden Perus. *Umschau* 1958, 645–681.
- Ghazoul, J., Liston, K.A., Boyle, T.J.B., 1998. Disturbance-induced density-dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. *J. Ecol.* 86, 462–473.
- Gionali, E., González-Teuber, M., 2005. Effect of support availability, mother plant genotype and maternal support environment on the twining vine *Ipomoea purpurea*. *Plant Ecol.* 179, 231–235.
- Harrison, S., Bruna, E., 1999. Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* 22, 225–232.
- Hendrix, S.D., Kyhl, J.F., 2000. Population size and reproduction in *Phlox pilosa*. *Conserv. Biol.* 14, 304–313.
- Heschel, M.S., Paige, K.N., 1995. Inbreeding depression, environmental stress, and population size variation in Scarlet Gilia (*Ipomopsis aggregata*). *Conserv. Biol.* 9, 126–133.
- Jordan, E., 1983. Die Verbreitung von *Polylepis*-Beständen in der Westkordillere Boliviens. *Tuexenia* 3, 101–116.
- Jung, K., Kaletta, K., Segner, H., Schüürmann, G., 1999. ¹⁵N metabolic test for the determination of phytotoxic effects of chemical and contaminated environmental samples. *Env. Sci. Poll. Res.* 6, 72–76.
- Kearns, C.A., Inouye, D.W., 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Colorado, USA.
- Kessler, M., 1995. *Polylepis*-Wälder Boliviens: Taxa, Ökologie, Verbreitung und Geschichte. *Diss. Bot.* 246. J. Cramer, Berlin, Stuttgart, Germany.
- Martin, F.M., 1959. Staining and observing pollen tubes by means of fluorescence. *Stain Technol.* 34, 436–437.
- Menges, E.S., 1991. Seed germination percentage increases with population size in a fragmented prairie species. *Conserv. Biol.* 5, 158–164.
- Purcell, J., Brelsford, A., Kessler, M., 2004. The world's highest forest. *Am. Sci.* 92, 454–461.
- Quesada, M., Fuchs, E.J., Lobo, J.A., 2001. Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree *Pachira quinata* (Bombacaceae). *Am. J. Bot.* 88, 400–406.
- Renison, D., Cingolani, A.M., Suarez, R., Menoyo, E., Coutiers, C., Sobral, A., Hensen, I., 2005. The restoration of degraded mountain woodlands: effects of seed provenance and microsite characteristics on *Polylepis australis* seedling survival and growth in Central Argentina. *Restor. Ecol.* 13, 1–9.
- Renison, D., Hensen, I., Suarez, R., Cingolani, A.M., 2006. Cover and growth habit of *Polylepis* woodlands and shrublands in the mountains of central Argentina: human or environmental influence? *J. Biogeogr.*
- Schmidt-Lebuhn, A.N., Kumar, M., Kessler, M., 2006. An assessment of the genetic population structure of two species of *Polylepis* Ruiz & Pav. (Rosaceae) in the Chilean Andes. *Flora* 201, 317–325.
- Schulz, H., Gehre, M., Hofmann, D., Jung, K., 2001. Nitrogen isotope ratios in pine bark as an indicator of N emissions from anthropogenic sources. *Env. Monitor. Assess.* 69, 283–297.
- Seltmann, P., Cocucci, A., Renison, D., Hensen, I., accepted a. Mating system, outcrossing distance effects and pollen availability in the wind-pollinated treeline species *Polylepis australis* (Rosaceae). *Basic Appl. Ecol.*
- Seltmann, P., Hensen, I., Renison, D., Wesche, K., Ploch, S., Dueñas, R.J., Cocucci, A.A., Jung, K., submitted b. Biparental inbreeding depression, genetic relatedness and progeny vigor in a wind-pollinated treeline species in Argentina. *Ann. Bot.*
- Sih, A., Baltus, M.S., 1987. Patch size, pollinator behavior and pollinator limitation in catnip. *Ecology* 68, 1679–1690.
- Simpson, B.B., 1979. *A revision of the Genus Polylepis* (Rosaceae: Sanguisorbeae). Smithsonian Institution Press, Washington, USA.
- Simpson, B.B., 1986. Speciation and specialization of *Polylepis* in the Andes. In: Vuilleumier, F., Monasterio, M. (Eds.), *High Altitude Tropical Biogeography*. Oxford University Press, Oxford, USA, pp. 304–316.

- Smouse, P.E., Sork, V.L., 2004. Measuring pollen flow in forest trees: an exposition of alternative approaches. *For. Ecol. Manage.* 197, 21–38.
- Stephenson, A.G., Quesada, M., Schlichting, C.D., Winsor, J.A., 1995. Consequences of variation in pollen load size. In: Hoch, P.C., Stephenson, A.G. (Eds.), *Experimental and Molecular Approaches to Plant Biosystematics*. Missouri Botanical Garden, St. Louis, USA.
- Sutter, K., Jung, K., Krauss, G.-J., 2002. Effects of heavy metals on the nitrogen metabolism of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. *Environ. Sci. Poll. Res.* 9, 417–421.
- Teich, I., Cingolani, A.M., Renison, D., Hensen, I., Giorgis, M., 2005. Do domestic herbivores retard *Polylepis australis* woodland recovery in the mountains of Córdoba, Argentina? *For. Ecol. Man.* 219, 229–241.
- Van Treuren, R., Bijlsma, R., Van Delden, W., Ouborg, N.J., 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* 66, 181–189.
- Weiner, J., Martinez, S., Müller-Schärer, H., Stoll, P., Schmid, B., 1997. How important are environmental effects in plants? A study with *Centaurea maculosa*. *J. Ecol.* 85, 133–142.