

Mating system, outcrossing distance effects and pollen availability in the wind-pollinated treeline species *Polylepis australis* BITT. (Rosaceae)

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Abstract

Fragmentation may negatively affect plant fitness through pollen limitation and increased levels of inbreeding. Effects of fragmentation may vary with regard to life form and breeding system, and few studies exist for wind-pollinated trees. We examined the effects of hand-selfing, varying outcrossing distances and pollen addition on seed mass and germination rate of *Polylepis australis* BITT. (Rosaceae), a wind-pollinated treeline species endemic to Argentina. We also investigated pollen germination on the stigma and pollen tube growth to determine compatibility resulting from selfing and outcrossing. Selfing reduced seed germination rates with significant differences between open pollination and outcrosses at 30 km. In addition, we found a tendency for pollen germination and pollen tube growth to decrease following selfing. Between-fragment crosses resulted in a trend of higher reproductive output than within-fragment crosses, whereas values were similar between open pollination and between-fragment crosses. Pollen addition did not increase reproductive success neither in small nor in larger fragments. Our results suggest that highly isolated *P. australis* forests have a potential for inbreeding depression through selfing and within-fragment crosses. However, the results also indicate that pollen flow between *P. australis* forest fragments is still effective at the current fragmentation level, counteracting negative effects on seed quality resulting from reproductive isolation.

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Zusammenfassung

Durch Habitatzerstörung und Fragmentierung isolierte und kleine Populationen leiden häufig unter erhöhter Inzuchtdepression und Pollenlimitierung, deren Ausmaß jedoch in Abhängigkeit von Lebensform und Fortpflanzungssystem variiert. In der vorliegenden Studie untersuchten wir den Einfluss von Hand-Selbstbestäubung, verschiedenen Kreuzungsdistanzen bis zu 30 km und Pollenzugabe auf Samenmasse und -keimung von *Polylepis australis* (Rosaceae), einer windbestäubten, in Argentinien endemischen Baumart der oberen Waldgrenze. Darüber hinaus analysierten wir den Einfluss von Selbst- und Fremdbestäubung auf Pollenkeimung und Pollenschlauchwachstum. Selbstbestäubung führte zu signifikant geringeren Keimraten im Vergleich zu natürlicher Bestäubung und Fremdbestäubung mit Pollen aus > 30 km Distanz. Auch fanden wir eine Tendenz zur Reduktion von Pollenkeimung und Pollenschlauchwachstum nach Selbstbestäubung. Kreuzungen zwischen Fragmenten führten zu höherem

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Reproduktionserfolg als Kreuzungen innerhalb von Fragmenten, während der Reproduktionserfolg aus natürlicher Bestäubung und aus Kreuzungen zwischen Fragmenten vergleichbare Werte aufwies. Weder in kleinen noch in großen Populationen führte die Zugabe von Pollen zu einer Erhöhung des Reproduktionserfolges. Unsere Ergebnisse machen deutlich, dass zwar in stark isolierten *P. australis* Wäldern die Gefahr für Inzuchtdepression besteht, der nach wie vor effektive Pollen- und Genfluss zwischen Waldfragmenten von *P. australis* jedoch negativen Isolationseffekten entgegenwirkt.

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Keywords: Central Argentina; Genetic incompatibility; Inbreeding depression; Outcrossing distances; Pollen addition; Wind pollination

Introduction

Theory predicts that limited dispersal of both seeds and pollen results in relatedness among near neighbors in natural populations, which makes mating with close relatives likely. In small and isolated fragments the limited number of mating partners and reduced levels of genetic variation may further increase the likelihood of inbreeding and inbreeding depression (e.g. Fischer & Matthies, 1997; Stacy, 2001), and probably reduce reproductive fitness (e.g. Fischer & Matthies, 1997; Hensen & Wesche, 2006; Lienert & Fischer, 2004; Ramula, Toivonen, & Mutikainen, 2007). While the potential for biparental inbreeding depression and the importance of outcrossing distances on reproductive output are well documented in insect-pollinated species (e.g. Lienert, Diemer, & Schmid, 2002; Paschke, Bernasconi, & Schmid, 2005; Stacy, 2001), information is still limited for wind-pollinated trees, especially angiosperms. In addition, limited pollen quantity in anthropogenically fragmented habitats has long been known to negatively affect reproductive output and offspring vigor in many animal-pollinated species (e.g. Larson & Barrett, 2000; Pflugshaupt, Kollmann, Fischer, & Roy, 2002), and some experimental evidence demonstrates the possibility of pollen limitation even in wind-pollinated trees (e.g. Allison, 1990a, 1990b; Holm, 1994; Karrenberg, Kollmann, & Edwards, 2002; Knapp, Goedde, & Rice, 2001).

Many forest habitats become ever more fragmented. Thus, decreased reproductive output and impaired offspring vigor as possible consequences of increased inbreeding depression, pollen limitation, and decreased interpopulation gene flow might become an increasing problem (Young, Boyle, & Brown, 1996). It is thus important to understand the mating system and the pollination biology of anthropogenically fragmented populations of a certain tree species. Here, we contribute to this general objective by analyzing the mating system of a wind pollinated treeline species of the *Polylepis* genus (Rosaceae, Sanguisorbeae) that comprises about 28 wind-pollinated species distributed from southern Venezuela to central Argentina (Schmidt-Lebuhn, Kessler, & Kumar, 2006). Mainly as a consequence of

pasturing, burning and agriculture over the past few centuries, the current range of *Polylepis* forests is characterized by a high level of fragmentation which led to their listing as one of the most endangered forest ecosystems worldwide (UNEP-WCMC, Monitoring World Conservation Centre, 2004). As previous observations have reported low pollen dispersal and centuries of anthropogenic impact (Fjeldså & Kessler, 1996) pollen limitation and inbreeding depression could be suspected in the *Polylepis* genus.

Here, we used *Polylepis australis* BITT., the southernmost *Polylepis* species, and specifically focused on the following questions: Is self-pollination possible? Are there differences in the reproductive output of self- and cross-pollinated flowers? Does the distance between mates affect reproductive output? Are there any indications for pollen limitation being a limiting factor in the pollination process? Answering these questions is important because fragmentation effects cannot be extrapolated from similar species as, to the best of our knowledge, the pollination ecology of no wind pollinated South American treeline species has been analyzed to date.

Materials and methods

Study area and species

The study was carried out in the Córdoba mountain range of central Argentina (N–S orientation, 31°34'S, 64°50'W). Mean temperatures of the coldest and warmest months at 2100 m a.s.l. are 5.0 and 11.4 °C, respectively (annual mean 8 °C), and mean annual precipitation is 840 mm (Cabido, Breimer, & Vega, 1987). The mountain range acts as a barrier to E–W or W–E winds and predominant wind directions are S–N or N–S (Colladon, 2002). The landscape is a mosaic of different types of tall tussock grasslands, pasture land, granite outcrops, exposed rock surfaces produced by soil erosion, and fragments of open-canopy forests dominated almost exclusively by *P. australis* trees (Cingolani, Renison, Zak, & Cabido, 2004), whose stands are located between 900 and 2.884 m a.s.l.

P. australis is an endemic treeline species of Central and Northwest Argentina with a maximum height of 10 m (Simpson, 1979). In Córdoba it flowers between September and November. Its racemiform pendulous inflorescences are produced annually and carry up to 12 wind-pollinated flowers with typical anemophilous features such as reduced inconspicuous corollas, protogyny and a large stigmatic surface area. Fruits are mostly single-seeded nutlets (hereafter referred to as seeds), which are enclosed in a turbinate and winged receptaculum and dispersed between mid-January and February. They germinate readily without any dormancy-breaking treatment (Renison & Cingolani, 1998). The results of preliminary investigations revealed that *P. australis* is characterized by high pollen viability (mean 86%), and an exceptionally long period of pollen longevity (47% of pollen still viable 5 days after pollen release; Seltmann unpubl.).

Test for self-fertilization and effect of outcrossing distances

In September 2002, we randomly selected 20 *P. australis* trees equally distributed between two forest fragments with a distance of 1 km between fragments (fragment 1: 1990 m a.s.l., fragment 2: 2220 m a.s.l., both composed of >1000 individuals). Fragments were chosen due to good comparability of habitat conditions to near-natural forests in the same region. Soil nutrients were lower in fragment 1, but within the natural variation found in well-preserved forests of the area (e.g. fragment 1: pH (H₂O) 4.7; total N 5345 ppm, P 32 ppm, Ca 73 ppm, K 10 ppm; fragment 2: pH (H₂O) 5.1; total N 12060 ppm, P 54 ppm, Ca 185 ppm, K 27 ppm).

We performed the following five treatments in each of the 20 trees: (1) No experimental manipulation (open pollination: OP). (2) Pollination with pollen from a different flower of the same plant ('hand self-pollination': HSP). (3) Pollination with a pollen mix from eight to 10 paternal trees from the same forest fragment; all paternal trees were within a distance of 20 m from the maternal tree (within-fragment crosses, 'hand cross-pollination 1': HCP-1). (4) Pollination with a pollen mix from eight to 10 paternal trees from the other forest fragment, respectively (distance 1 km; crosses between closely separated fragments, 'hand cross-pollination 2': HCP-2). (5) Pollination with a pollen mix from eight to 10 paternal trees from another forest fragment 30 km south of the two study fragments (fragment 3; 2040 m a.s.l., >1000 individuals; pH (H₂O) 4.9, total N 6180 ppm, P 29 ppm, Ca 78 ppm, K 13 ppm; crosses between widely separated fragments, 'hand cross-pollination 3': HCP-3).

For each tree and treatment, we selected six inflorescences with flowers at the budding stage. Inflores-

cences were selected within reach but at random heights and expositions. Inflorescences were labeled using ribbons with specific colors for each pollination treatment. To accomplish hand pollination, we collected flowers in the male phase and carefully applied pollen to the receptive stigma using the anthers themselves as pollen applicators. Care was taken to ensure that stigmas were always pollen saturated and that pollen was not dispersed to non-target inflorescences.

Due to variability in number of flowers per inflorescences, this strategy resulted in a mean of 24 hand-pollinated flowers per tree and treatment (range: OP 21–32 flowers; HSP 19–23 flowers; HCP-1 20–31 flowers; HCP-2 20–32 flowers; HCP-3 20–31 flowers).

After selection, experimental inflorescences were bagged with pollen-proof bags (glassine shoot bags, Seedburo Equipment Company, Chicago), while open pollination inflorescences were left open until the receptive period was over. Five to 7 days later, when flowers were in the receptive female phase, bags were removed carefully, hand pollinations were performed, and inflorescences were subsequently enclosed in the same bag. Although flowers in the hand pollination treatments were not emasculated, the protogyny of the flowers made it likely that stigmas received handself or outcross pollen before being exposed to passive self-pollen. Pollination bags were removed in October 2002, and replaced with nylon bags to protect fruits and avoid fruit loss due to early dispersal. Nylon bags were also applied to the OP treatment. All fruits were collected in January 2003.

Pollen germination and pollen tube growth: self- and cross-compatibility

An additional hand pollination experiment was conducted in the laboratory to determine compatibility resulting from selfing and outcrossing. We investigated pollen germination on the stigma and pollen tube growth resulting from hand self-pollination using pollen from a different flower of the same plant (with 13 flowers serving as females, eight flowers serving as males; $n = 5$ trees), as well as hand cross-pollination with pollen from the same forest fragment (10 flowers serving as females ($n = 5$ trees), six flowers serving as males ($n = 5$ trees). Again, anthers themselves were used as pollen applicators, resulting in a single-donor flower per receptive flower. Ninety-six hours after pollen application, flower styles were fixed in 70% ethanol and then softened in 5 M KOH for 1 h. Thereafter, styles were rinsed and stained and the total number of pollen grains on the stigma, the number of germinated pollen grains and the number of pollen tubes reaching the style were counted using the aniline blue technique and epifluorescence microscopy (Kearns & Inouye, 1993). It was not possible to count pollen tubes at the base of the style because tubes within the style were not distinguishable.

Pollen addition

Pollen addition (hand pollination with surplus pollen, PA) was performed to assess whether the amount of pollen reaching stigmas acts as a constraint on the reproductive output of *P. australis*. Pollen addition was performed during the main flowering season (October) of the years 2002 and 2003. In 2002, we simultaneously employed pollen addition in four forest fragments differing in size (fragment I: >1000 trees (= fragment 1, see above), fragment II: ~200 trees, fragment III: 29 trees, fragment IV: 3 trees; fragment I located ~35 km to the north of fragment II–IV; the latter fragments are located between 2040 and 2170 m a.s.l., with a distance of at least 2 km between them). We randomly chose 10 trees per fragment (except for fragment IV where we used all three trees). On each tree, we selected six inflorescences for the pollen addition treatment (PA) and six for the control (open pollination; OP), resulting in a mean of 29 flowers per tree and treatment in the year 2002 and 30 in the year 2003 (range: OP 22–35 flowers; PA 19–32 flowers). In 2002, pollen addition by hand included a pollen mix from eight to ten trees (three trees in the case of fragment IV) of the same forest fragment. In 2003, we repeated the treatment in the two smaller fragments (fragments III and IV), and used a pollen mix from eight to 10 trees from another forest fragment at least 1 km away. Fruits were protected by nylon bags in November (2002 and 2003) and collected in January the next year.

Determination of seed mass and germination rate

The collected seeds of all pollination treatments (total sample sizes between 422 and 534, Fig. 1) were stored under ambient laboratory conditions for 1 month. Half of the seeds per tree and treatment were selected randomly and then weighed individually to the nearest 0.1 mg in the laboratory. Seed germination was tested using all collected seeds without any other pretreatment 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 50 days, when germination was zero.

Statistical analysis

Most statistical analyses of the data were performed using SPSS 12.0 (SPSS Inc., 2003) and R, version 2.2.1 (The R Foundation for Statistical Computing, 2005). Germination data were arcsin squareroot transformed and seed mass was ln-transformed to achieve normal distribution of residuals. In order to contrast seed mass and seed germination rates resulting from the tests for self-fertilization and effects of outcrossing distances, a

nested mixed ANOVA was performed with the fixed factor “pollination treatment” nested within the random block factor “tree” and “tree” nested in the random block factor “fragment”. Bonferroni corrected paired *t*-tests further analyzed mean differences between the groups ($p \leq 0.005$; two-tailed significance level). In order to contrast seed mass and seed germination rate resulting from open pollination and pollen addition for different fragment sizes, a two way repeated measures ANOVA was performed with pollination treatments as within-subject variables and forest fragments as between-subject factor terms. A *t*-test explored the reproductive output of 2002 and 2003 for fragments III and IV in the pollen addition experiment. Proportions of germinated pollen grains and pollen tubes were

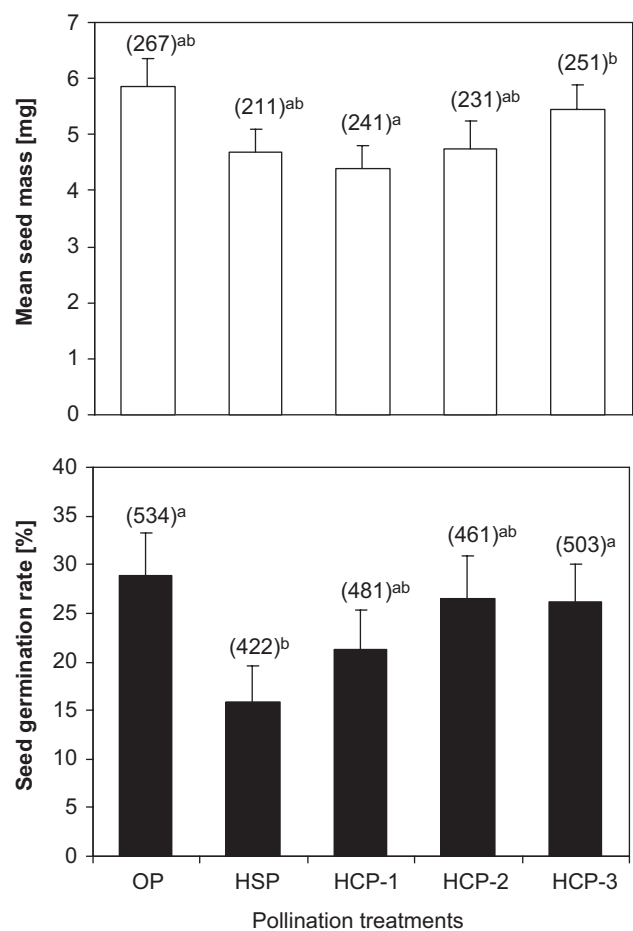


Fig. 1. Seed mass and seed germination rate (means + SE) per *Polylepis australis* tree resulting from the different pollination treatments. Different letters indicate significant differences (paired *t*-tests, $p \leq 0.005$ after Bonferroni correction); $n = 20$ trees per pollination treatment; total number of seeds per treatment are shown in parentheses. Treatments: OP, open pollination; HSP, hand self-pollination; HCP-1, hand cross-pollination (crosses within a fragment); HCP-2, hand cross-pollination (1 km, crosses between closely separated fragments); HCP-3, hand cross-pollination (30 km, crosses between widely separated fragments).

compared between self- and cross-pollinated flowers with Pearson's χ^2 -test for homogeneity, and a power analysis was performed using PASS (2002).

Results

Open and self-fertilization, and the effect of outcrossing distances (Table 1)

For open pollination, mean seed mass per *P. australis* tree ranged from 3 to 11 mg (mean 5.9 mg, SE 0.49, Fig. 1) and germination rate from 3% to 79% (mean 28.9%, SE 4.4; Fig. 1). Self-fertilization was possible, but selfed mean seed mass was 20% lower than mean seed mass of open pollination ($p = 0.02$; n.s. after Bonferroni correction) and mean seed germination rate was 55% lower ($p = 0.004$; Fig. 1).

There was a constant increase of mean seed mass with increasing outcrossing distance (Fig. 1). The difference of 19% between seed mass resulting from long-distance outcrosses (HCP-3) and that of seeds from flowers pollinated with pollen from the same forest fragment (HCP-1) was statistically significant after Bonferroni correction ($p = 0.002$). Between-fragment crosses resulted in a non-significant trend for higher seed germination rates, compared with within-fragment crosses (Fig. 1).

Pollen germination and pollen tube growth: self- and cross-compatibility

Pollen germination and pollen tube growth generally showed lower values following self-pollination than

cross-pollination: cross-pollen germination on the stigma was $54.8 \pm 12.2\%$ (mean \pm SE) while self-pollen germination was $45.4 \pm 9.0\%$ (mean \pm SE). Concordantly, $20.6 \pm 9.9\%$ (mean \pm SE) of the cross-pollen tubes and $10.9 \pm 7.7\%$ (mean \pm SE) of the self-pollen tubes reached the style. In both cases, these differences were not significant (Pearson's χ^2 -test, Table 2). However, the results of a power analysis indicate that our sample size of $n = 165$ achieved only 22% power to detect an effect size of 0.1 with $\alpha = 0.05$ in the case of pollen germination and 38% power in the case of pollen tube growth (Table 2).

Pollen addition

We found significant differences in natural reproductive output between the four forest fragments in 2002 (included as between-subjects factor, Table 3), as well as between study years in fragment 3 (t -test; seed mass: $p < 0.01$ and seed germination rate: $p < 0.01$). However, pollen addition did not have a significant effect on the reproductive output of *P. australis* trees in any of the study years (Table 3, Fig. 2). Furthermore, the treatment was not affected by the forest fragment (added as interaction term, Table 3). This implies that pollen addition did not lead to a significant increase in mean seed mass or seed germination rate in any of the fragments.

Discussion

In our experiments, we found decreased seed germination rates following selfing in *P. australis*. There are

Table 1. Nested mixed ANOVA for effects of pollination treatments ($n = 20$ trees per pollination treatment: open pollination, hand self-pollination, hand cross-pollination within a fragment, hand cross-pollination between closely separated fragments, hand cross-pollination between widely separated fragments) on seed mass per tree and seed germination rate in *Polylepis australis*

Estimate of reproductive output	Source	df	<i>F</i>	<i>P</i>
Seed mass per tree (mg)	Fragment	1	1.31	0.32
	Tree (fragment)	18	1.53	0.10
	Treatment (tree [fragment])	4	4.12	0.00
Seed germination (%)	Fragment	1	0.26	0.62
	Tree (fragment)	18	1.52	0.10
	Treatment (tree [fragment])	4	3.52	0.01

Each data point in the analysis is (a) the mean seed mass per tree and (b) the germination percentage per tree (see also Fig. 1).

Table 2. Pearson's χ^2 -tests for homogeneity in *Polylepis australis* pollen germination and pollen tube growth (pollen tubes reaching the style) between self-pollinated and cross-pollinated flowers ($n = 165$ pollen grains), and the corresponding power analysis (Power and β)

	χ^2	df	Effect size (W)	<i>P</i>	Power	β
Pollen germination	1.38	1	0.09	0.24	0.22	0.78
Pollen tubes reaching the style	2.74	1	0.13	0.10	0.38	0.62

Table 3. Two way repeated measures ANOVA for the effect of pollen addition (compared to open pollination) on seed mass per tree and seed germination rate for *Polylepis australis* fragments differing in size in 2002 (four forest fragments) and 2003 (only the two smaller fragments)

Estimate of reproductive output	Source	df	F	P
<i>Year 2002</i>				
Seed mass per tree (mg)	Pollination treatment	1.00	0.31	0.59
	Forest fragment	3.00	11.98	0.00
	Pollination treatment*forest fragment	3.00	0.30	0.83
Seed germination (%)	Pollination treatment	1.00	1.80	0.19
	Forest fragment	3.00	4.19	0.01
	Pollination treatment*forest fragment	3.00	0.52	0.67
<i>Year 2003</i>				
Seed mass per tree (mg)	Pollination treatment	1.00	0.29	0.60
	Forest fragment	1.00	0.85	0.38
	Pollination treatment*forest fragment	1.00	0.16	0.69
Seed germination (%)	Pollination treatment	1.00	0.09	0.77
	Forest fragment	1.00	1.00	0.34
	Pollination treatment*forest fragment	1.00	0.94	0.35

Fragments I–III: $n = 10$ trees per treatment and fragment; fragment IV: $n = 3$ trees per treatment and fragment. Each data point in the analysis is (a) the mean seed mass per tree and (b) the germination percentage per tree (see also Fig. 2).

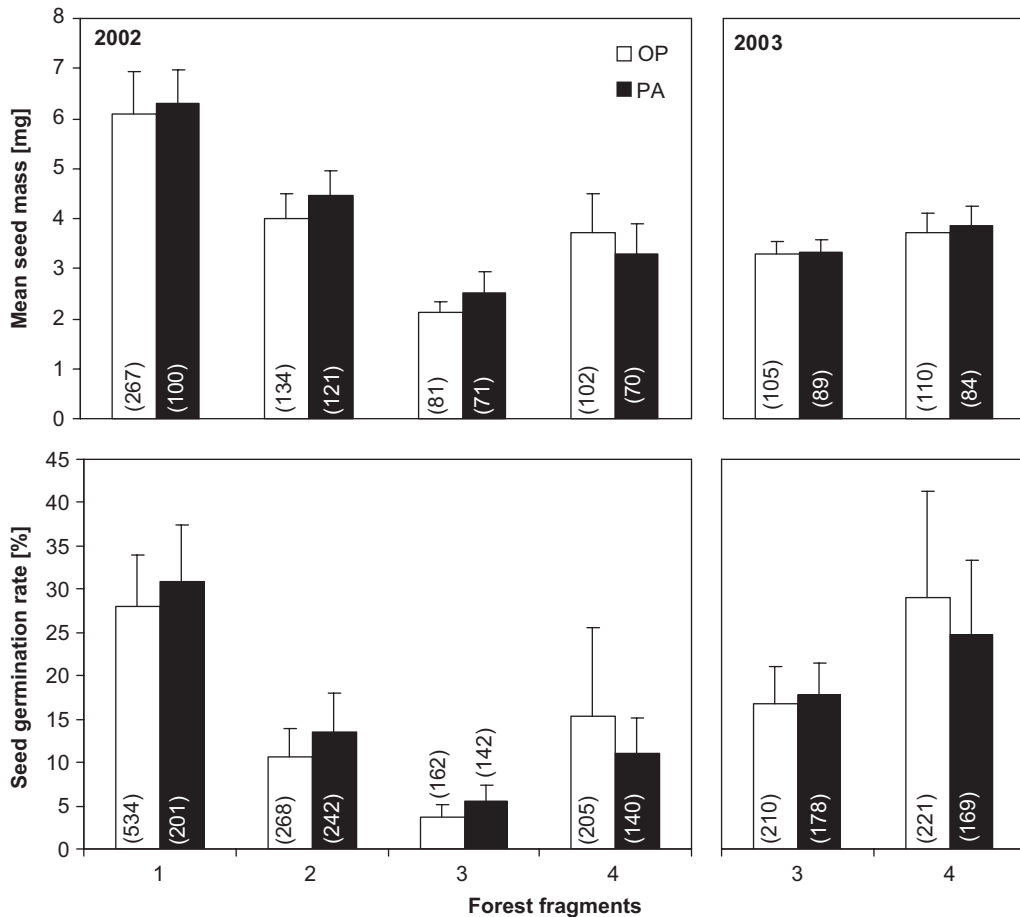


Fig. 2. Mean seed mass and seed germination rate (means + SE) per *Polylepis australis* tree in 2002 and 2003 after open pollination (OP) and pollen addition (PA) in forest fragments of differing size. Numbers in the x-axis indicate fragment sizes of > 1000 trees (I), ~200 trees (II), 29 trees (III) and 3 trees (IV). (I–III: $n = 10$ trees; IV: $n = 3$ trees; total number of seeds per treatment are shown in parentheses).

two possible explanations: (1) gametophytic self-incompatibility mechanisms that delay or prevent germination and tube growth of self-pollen, and (2) inbreeding depression. The fact that we found a tendency for a decrease of pollen germination and of the number of pollen tubes reaching the style in selfed flowers speaks in favor of the first possibility, whereby self-incompatibility would not be complete. Further support for this interpretation is the repeated observation of the presence of gametophytic self-incompatibility systems in the Rosaceae family (e.g. Ishimizu, Shinkawa, Sakiyama, & Norioka, 1998; Ushijima et al., 2003). However, the high variability we noted in seed germination rates among individuals following selfing suggests that *P. australis* does not possess a classical self-incompatibility system as defined by Nettancourt (1977), because such systems exclude selfing by preventing the germination of pollen grains or by arresting pollen tube growth high in the style (Gigord, Lavigne, & Shykoff, 1998; Nettancourt, 1977). In contrast, inbreeding depression might result in variable seed production upon selfing as different individuals may vary in the number of lethal equivalents they carry. In this case, reduced seed germination rate would be the result of early acting inbreeding depression, with an increase in the proportion of recessive alleles in homozygous condition (Gigord et al., 1998; Reinartz & Les, 1994). However, it is generally difficult to assess the relative role of genetic incompatibility and inbreeding depression in explaining the failure of selfing (Charlesworth, 1985).

Our finding of increased reproductive success in crosses between fragments compared to within-fragment crosses suggests spatial genetic structuring in *P. australis*. Thus, the average degree of relatedness of parent plants may have been lower for parents of between-fragment crosses than for within-fragment crosses. However, this assumption needs further verification by additional data as our result was only significant in the case of seed mass.

However, a likely explanation for the assumption of spatial genetic structuring is the restricted seed dispersal of *P. australis*. Seeds are reported to be wind dispersed (Simpson, 1979, 1986), but personal observations suggest that a high proportion of seeds is dispersed by gravity alone. Reduced seed mass and seed germination rate resulting from crosses within a fragment may either be caused by genetic incompatibility or by biparental inbreeding depression in cases where trees are crossed with relatives. Again, reproductive output resulting from within-fragment crosses was highly variable among the maternal trees; an effect that may result from differences in the genetic relatedness of trees within a forest fragment.

In our experiments, reproductive output resulting from hand outcrosses with pollen from varying distances

was lower than that of open pollinated flowers, although there were no significant differences in most cases. This could be due to any, however slight, effects of bagging on seed development, or could be due to the fact that hand pollination was performed just once over the flowers female phase, whereas open-pollinated flowers received pollen over a more prolonged period. On the other hand, these differences may also indicate that limited pollen quantity does not play an important role in *P. australis* reproduction. In accordance, the results of the pollen addition experiment demonstrated that pollen availability did not limit reproductive success in any of the fragments in both study years. This result may have been affected in 2002 by the use of pollen from the same forest fragment for pollination manipulations. However, the same results were extrapolated in the second year of investigation using pollen at least 1 km away. This suggests that all trees received a sufficient amount of pollen grains for pollination. Our data are in line with Dow and Ashley (1996, 1998) and Streiff et al. (1999), who found high gene flow between populations of different *Quercus* species, but differ from the results of Allison (1990a), Holm (1994), and Knapp et al. (2001) who found evidence for limited pollen quantity in wind-pollinated trees. However, as there were significant differences in seed mass and seed germination rate between the studied forest fragments and years in the pollen addition experiment, despite unlimited pollen availability, we assume that *P. australis* seed quality is mainly affected by environmental factors such as habitat degradation or climatic differences between years. This suggestion is in accordance with Renison, Hensen, and Cingolani (2004), who found that anthropogenic soil degradation affects seed viability in *P. australis*.

The fact that we found no differences in reproductive output between open pollination, pollen addition and hand long-distance crosses speaks in favor of effective pollen flow between forest fragments. Thus, our data are in contrast to Salgado-Labouriau (1979), and Fjeldså and Kessler (1996) who report that pollen dispersal in *Polylepis* is very limited, but in line with recent genetic studies supporting extensive gene flow in this genus (Schmidt-Lebuhn, Kumar, & Kessler, 2006). In accordance, ongoing studies of Seltmann, Renison, & Schurr (unpublished) revealed pollen dispersal distances of *P. australis* ranging up to 80 km from the nearest population. Furthermore, *P. australis* pollen grains remain viable for an exceptionally long time, compared to other wind-pollinated species (e.g. Huang, Zhu, Mu, & Lin, 2004; Pacini, Franchi, Lisci, & Nepi, 1997; Wang, 2004) and thus long enough to be able to complete the pollination process even over long distances. Thus, our data contribute to the general assumption of extensive pollen flow in wind-pollinated trees (e.g. Hamrick & Nason, 2000), making them more resistant to reproductive isolation through forest fragmentation.

We conclude that *P. australis* forests are currently not affected by pollen limitation and increased levels of inbreeding. However, the reduced reproductive output through selfing and within-fragment crosses may be of importance in completely isolated fragments and solitary trees. As stands and individuals become increasingly more isolated due to ongoing land use practices, neighbourhood sizes might decline to the point where sufficient pollen will not be available to some individuals, leading to reproductive failure (Koenig & Ashley, 2003). Therefore, a clear conservation strategy is urgently needed to avoid complete isolation of *P. australis* fragments.

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