Ladd, P.G. and Arroyo, M.T.K. (2009) *Comparisons of breeding systems between two sympatric species, Nastanthus spathulatus (Calyceraceae) and Rhodophiala rhodolirion (Amaryllidaceae), in the high Andes of central Chile.* **Plant Species Biology**, 24 (1). pp. 2-10.

Comparisons of breeding systems between two sympatric species, Nastanthus spathulatus (Calyceraceae) and Rhodophiala rhodolirion (Amaryllidaceae), in the high Andes of central Chile

Philip G. Ladd[1] and Mary T.K. Arroyo[2]

[1]School of Environmental Science, Murdoch University, Murdoch, Western Australia 6150, Australia

[2]Institute of Ecology and Biodiversity, Faculty of Science, University of Chile, Casilla 653, Santiago, Chile

Abstract

Alpine vegetation occurs over a wide range of ecological conditions. Thus, the breeding systems of alpine plants are likely to be diverse and vary from one geographical area to another. The reproductive characteristics of *Nastanthus spathulatus* (Calyceraceae) and *Rhodophiala rhodolirion* (Amaryllidaceae), species with contrasting floral morphology, were studied in the high Andes of Chile, which has a Mediterranean-type climate. Natural and supplemental open pollination, and cross pollination and self-pollination trials were carried out in the field. Flower visitors were quantified by field and video observations. Both species had high outcrossing properties, and *Nastanthus* was strongly self-incompatible. *Rhodophiala* could form some seed by self-pollination, but fruit and seed sets were much lower after self-pollination compared with outcrossing. The phenology and flower/inflorescence forms of

these species supported the view that alpine flowers are comparatively long lived and that the floral display contributes to a large proportion of the plant biomass. *Rhodophiala* was well attended by a native bee species (*Megachile sauleyi*) that was appropriately sized for efficient pollination. Although no flower visitors were observed on *Nastanthus* and wind pollination was discarded experimentally, a high proportion of the flowers produced seeds under natural pollination. Therefore, the seed set was not severely pollen limited in these species. Including previously published information, breeding systems are now known for 12 species on this Mediterranean alpine site and current knowledge suggests an emphasis on outcrossing breeding systems.

Introduction

Flower visitation by pollinators in the alpine environment can be notoriously erratic, both spatially and temporally (Totland 1994; Bergman *et al.* 1996; Arroyo *et al.* 2006), and overall community flower visitation rates are known to decline with increasing altitude from the lower alpine belt upward at three latitudes (subtropical, Mediterranean and Patagonian) in the South American Andes because of adverse weather conditions and cold temperatures (Arroyo *et al.* 1985; Arroyo & Squeo 1990). Flower visitation is influenced by both biotic and abiotic factors. In terms of biotic offerings, there is often a lack of resources as a result of small plant size and hence overall fewer flowers than would be produced in plants that are able to grow to a larger size. In addition, the flow rates of nectar are probably slow. Flowers that offer

nectar might be visited by different taxa than those that only offer pollen. Pollen flowers will be frequented more by bees that need pollen for provisioning larvae rather than flies or lepidopterans and thus might be less reliably pollinated as a result of a lower diversity of visitor types. Under such conditions different groups of plants have developed a range of strategies to ensure adequate seed set.

In the search for models to explain reproductive behaviour in the alpine zone, a number of hypotheses have been put forward to focus study on alpine plant breeding systems. An increased reliance on selfing has been suggested as a modification to ensure reliable seed set (autogamous assurance hypothesis; Mosquin 1966; Billings 1974). Shifting from a reliance on animal pollen vectors to wind pollination is another strategy (Arroyo & Squeo 1987; Linder 1998; Anderson 2000). Increasing flower longevity (Arroyo *et al.* 1982; Rathke 2003) and floral display in relation to total plant biomass (Fabbro & Körner 2004) have also been suggested and have been shown to increase the likelihood of pollination owing to more time being available for visitation and increased attractiveness (increased pollination probability hypothesis). In accordance with the latter, some outcrossing species show no depression in seed set with increasing altitude (Bingham & Orthner 1998). Despite demonstrated cases of autogamy, a growing body of alpine reproductive biologists (Arroyo & Squeo 1990; Körner 1999) considers that outcrossing breeding systems are relatively frequent in alpine areas. A high investment in flowers in relation to total plant biomass in many alpine plants supports this view (Fabbro & Körner 2004), although this is in contrast to the views of Molau (1993) who felt that the majority of arctic plants allocated 'a minor proportion of their available resources to regeneration'. Studies examining a suite of seven snow-bed species in the Austrian alps showed a full range of breeding systems from virtually complete outcrossing to

complete selfing (Scheffknecht *et al.* 2007), whereas heterostylous taxa, such as *Primula*, that have many alpine species are always outcrossing (e.g. Shimono & Washitani 2007). A study of over 137 tundra species from the Arctic alpine proposed two main life-history strategies (Molau (1993)). 'Pollen-risking' was detected in species that flower soon after the seasonal thaw and 'seed-risking' was observed in species that flower later in the season and are at risk of reduced seed set owing to an early onset of cold conditions.

Alpine vegetation occupies 3% of the globe's land area (Körner 1999), occurs on all continents and can be found from tropical to polar latitudes. Alpine regions provide one of the best replicated large-scale natural experimental areas that exists for ecological and macroecological work. Related taxa that span an altitudinal range can be examined to investigate patterns in reproductive behaviour with altitude (Totland 2001; Blionis & Vokou 2005). Although temperature is undoubtedly a main driver of many ecological processes in alpine areas, different alpine areas show large variations in growing season precipitation, cloudiness and wind conditions. Locally, all of these factors should affect pollinator activity as much as temperature. Moreover, alpine flora congregates numerous evolutionary lineages that are found in a wide variety of subtending vegetation types. Consequently, to obtain a global picture of the breeding systems and pollen limitations in alpine areas, far more experimental breeding system studies are needed to increase geographical and, in particular, taxonomic coverage. Thus, the present study assesses the breeding systems and seed set of two alpine species growing sympatrically in the Andes of central Chile. We selected these species because of their contrasting floral morphologies and different familial status. Our main aim was to determine how these species accord with contrasting hypotheses regarding the breeding systems of plant species in an alpine environment. We also provide information

on seed set and population density to assess the ability of these species to replace the existing populations of adults.

Methods

Study site

Studies of the pollination and reproductive properties were conducted at sites in the Chilean Andes in the La Parva-Valle Nevado area from 2300–3200 m a.s.l. at approximately $33^{°}$S. Plants commonly grew on steep slopes facing west to southwest, where their altitudinal ranges overlapped. The altitudinal range of *Nastanthus spathulatus* is reported to be 900–2200 m a.s.l.

http://www.chileflora.com/Florachilena/FloraSpanish/HighResPages/SH0033.htm; accessed September 2008), but it occurs up to 3200 m in the La Parva area, whereas *Rhodophiala rhodophiala* occurs from 1800 to 3200 m (Hoffmann *et al.* 1998; M. Arroyo, unpubl. data, 2007). In the La Parva area, *Nastanthus* is common at the higher end of the altitudinal range and grows with *Rhodophiala* at site 1, whereas only *Rhodophiala* grows at a lower altitude (2300 m) at site 2.

Study species

Nastanthus spathulatus (Phil.) Miers. (Calyceraceae) is a rosette herb that produces numerous densely arranged heads of flowers on mature plants that are similar to some species in the Asteraceae, with which the Calyceraceae forms a monophyletic group (Bremer & Gustafsson 1997; Fig. 1). The central capitulum is generally much larger (bears more flowers) than the peripheral capitula. The florets are densely packed on each capitulum, are greenish in color,

have no scent, offer no nectar and at anthesis the pollen is displayed as a khaki-colored mass on a pollen presenter approximately 3–4 mm above the corolla lobes. The inferior ovary has only one ovule and the pollen : ovule ratio is 868 (Table 1). Maturity of the inflorescences is centrifugal, and the anthesis of individual flowers within inflorescences has no specific pattern. Inflorescences take approximately 9 days from the opening of the first flower to the opening of all flowers. Flowers open initially by separation of the tepals and the style bearing the pollen mass takes approximately 4 h to become fully elongated. However, it takes 9 days from the first flower opening for the stigmas to swell to twice their diameter at anthesis. Removal of pollen from the presenter was variable, in some cases it was removed in 2 days, but in other cases it remained for over 9 days. In their general survey of flower visitors, Arroyo *et al.* (1982) reported ants as visitors to the inflorescences of this species in the central Chilean Andes.

Rhodophiala rhodolirion (Baker) Traub. (Amaryllidaceae) is a perennial geophyte that flowers after the leaves have senesced (Fig. 2). There is one large white to pale pink (usually with pink to maroon spots in the corolla throat) showy flower per peduncle. The flowers have no scent, offer no nectar and have six stamens in two whorls (one long, one short) displayed prominently in the throat of the corolla. The style is curved below the stamens, has a papillate trilobed stigma, and is exerted beyond the anthers. There are three loculi with 8–22 ovules in the inferior ovary. The anthers contain a large number of pollen grains, but this is highly variable, and the pollen : ovule ratio is 6481 (Table 1). Flowers are open for 7.7 ± 0.2 days ($n = 7$) (mean $\pm$ standard error), and on the flowers that were followed for phenology the flowers took 3.2 ± 0.5 days ($n = 7$) to acquire pollen on the stigma. Most (but not all) stigmas

produce fluid in 4.3 ± 0.9 days ($n = 7$). Arroyo *et al.* (1982) reported that the flowers were visited by a *Megachile* species (under *Rhodophiala montana* Phil.).

Plant density

The density of the plants was assessed using the T-square method (Krebs 1989) and 30 random points. The whole population was censused for *Nastanthus*, whereas only *Rhodophiala* reproductive plants could be located so the census was of flowers, which was taken to be representative of the reproductive population. Groups of flowers were considered to be the same genet and to be separate from other plants if there was more than 5 cm between the peduncles. There was normally no difficulty in recognising separate plants.

Pollinator observations

The area in which *Rhodophiala* grew was patrolled for 1 h each day for 8 days at a slow continuous walk and the insects visiting the flowers were recorded. Observations were generally between 13.00 and 16.00 hours, when temperatures were 20–23°C and the sky was clear. A 1-h video was recorded on each day of suitable weather of either a *Nastanthus* plant with mature flowers or a group of *Rhodophiala* flowers at the same time as the flower visitor observation. The video was replayed and the visits of any insects were timed. Insect visits were very brief and the periods were derived from the mean of three stopwatch records of the video after rewinding each time. Insects captured at *Rhodophiala* were examined for pollen load and location of pollen on the insect.

Pollination treatments

In *Nastanthus*, individual capitula were used as the experimental units owing to the size of the small, tightly packed flowers. The following treatments were conducted: (i) open pollination without intervention; (ii) open pollination with artificial cross pollination; (iii) spontaneous self-pollination in which inflorescences were covered with wire mesh (1 mm opening) and gauze (0.2 mm opening); (iv) wind pollination in which inflorescences were emasculated and covered with wire mesh; and (v) inflorescences covered with wire mesh without emasculation were also prepared to match the wind pollination treatment. Five days after the treatments were set up, a sample of flowers was harvested and the stigmas were examined for pollen tubes under fluorescence microscopy. Other flowers on the same inflorescence were left to mature to investigate fruit set.

In *Rhodophiala*, individual flowers were used as the experimental units. The following treatments were conducted: (i) open pollination without intervention; (ii) open pollination with artificial cross pollination; (iii) stigma clogging where self pollen was applied to the stigma of a newly opened flower and cross pollen was applied 1–2 days later; (iv) spontaneous self-pollination where flowers were bagged with no other intervention; (v) self-pollination where flowers were bagged and self pollen was applied to the stigma; and (vi) cross pollination in which all flowers were emasculated and bagged until cross pollen was supplied and cross pollen was always from a plant more than 2 m distant from the recipient. Flowers were left in the field to mature and harvested before the capsules shed seeds. Seed set was determined by dissection of the ovary. Seed set results were analysed using non-parametric Kruskal–Wallis and Mann–Whitney U-tests because the data did not conform to parametric test requirements.

To quantify the stigmatic pollen load under natural pollination a further 20 flowers were harvested at random on 3 days (separated by 1 day on each occasion) and the pollen grains on the stigmas were counted under 100× magnification.

Natural seed set

In *Nastanthus*, four inflorescences with mature fruits were sampled at random from individual plants from the whole area to assess the seed set under natural conditions. A smaller sample of fruits was haphazardly collected at Portillo, approximately 50 km north, at a similar elevation. Seed set was determined by dissecting the fruits.

Results

Plant density and population structure

The density of *Rhodophiala* was twice that of *Nastanthus* (Table 1). Because the census of *Rhodophiala* was carried out only for flowering plants, the number of individuals was much higher than that of *Nastanthus*. The population structure of *Nastanthus* was skewed towards small, non-reproductive individuals, and only 10% of plants in the census were fertile. Similarly, the population structure of *Rhodophiala* was highly skewed towards plants with only one flower, and the mean number of flowers per plant was 3.4 ± 0.5 ($n = 60$) at site 1 and 1.6 ± 0.2 ($n = 60$) at site 2. After fruiting, *Nastanthus* plants died and the species appears to be monocarpic.

Pollinator observations

No insects were observed on *Nastanthus* flowers. During the 1-h observation period over 8 days between 800 and 850 *Rhodophiala* flowers were observed on each occasion and 3.8 ± 1.3 ($n = 8$) visits of *Megachile sauleyi* Guerin-Meneville were observed per hour and only one foraging occasion for a small, unidentified black bee. The foraging behaviour of *Megachile* was inconsistent; often bees were observed on flowers, flying away quickly, returning, perhaps alighting, and only visiting a small proportion of flowers if the flowers were clumped. Bees approached the corolla from the front and grasped the anthers. They did not always touch the stigma as an approach to the anthers from the side of the flower could avoid contact with the stigma.

Video filming covered 858 flowers over 11 h. For seven of the recorded hours, there were no bee visits. Only three foraging occasions and two observation occasions were recorded on *Rhodophiala* flowers for *M. sauleyi* and one foraging occasion for the small black unidentified bee. *Megachile* spent 1.6 ± 0.3 s on a flower, whereas the black bee spent 3.0 s foraging.

Two individuals of *M. sauleyi* were captured on *Rhodophiala* flowers. The individuals were approximately 14 mm long and pollen was observed on the lower part of the abdomen in dense hairs. On the two bees that were captured there were two main types of pollen—from *Rhodophiala* and *Phacelia secunda (Hydrophyllaceae)*. The *Rhodophiala* pollen was much larger than that of *Phacelia*, but was only 19 and 2% of the pollen load, respectively.

Pollination and seed production of *Nastanthus*

Pollen tubes were never recorded in the spontaneous self-pollination treatment, indicating the sporophytic incompatibility system in *Nastanthus*. Pollen tubes were consistently observed on stigmas of the open pollination and open pollination with supplemental cross-pollen treatments (Table 2). Pollen tubes were observed on stigmas that were both fully expanded and on those that were minimally expanded. In the mesh-enclosed and emasculated treatment, no pollen tubes were found. In the two examples of the mesh-enclosed without emasculation treatment, one stigma out of the 11 examined showed pollen-tube growth. In these inflorescences, however, a few stigmas were pushed out of the mesh enclosure as the style elongated. This means that not all of the stigmas had been enclosed for all of the time. Thus, the deposition of germinable pollen grains in the mesh-enclosed treatment might be an artifact.

Fruit development appeared to occur without seed set in this species, but fruits without developing embryos were smaller than fertile fruits. There is no evidence of an apomictic breeding system in this species because flowers did not develop seeds when the styles were removed before anthesis. In the open pollination treatment, at least 15 and usually more than 20 flowers were retained so fruit set for those individual flowers could be recorded. Of these, 0.76 ± 0.08 flowers produced seeds, whereas in the open pollination with supplemental cross pollen, 0.86 ± 0.08 were fertile (Table 2). The autogamous treatment had a seed set of 0.02 ± 0.01, derived from only two of nine inflorescences examined. There was no seed set in the mesh-enclosed treatment.

Natural seed set in *Nastanthus* assessed in the middle of the flowering season at La Parva was very high (0.92 ± 0.01; Table 2). Predation of the inflorescences was an important impediment to seed production, with 61% of inflorescences showing some invertebrate damage and 10% of fruits destroyed. As the fruits were not completely mature at the time of harvest, the degree of damage would be higher at the time of seed dispersal. However, the estimated range of seed production for site 1 calculated from the 95% confidence intervals of reproductive plant density was 6.5–19.5 seeds/m^2, considerably higher than the present estimated plant density (Table 1).

Pollination and seed production of *Rhodophiala*

Natural pollination for *Rhodophiala* over 3 days showed that 65–75% of stigmas received more than 50 pollen grains and in all cases some pollen grains produced pollen tubes. At both sites only 5% of flowers were devoid of pollen. A number of stigmas also had pollen grains from other species. In particular, *Phacelia secunda* and Asteraceae pollen grains were detected on several occasions.

The removal of tagged *Rhodophiala* flowers by wandering cattle and/or horses was severe. At least 60% of the flowers at site 1 and 80% of the flowers at site 2 were destroyed. However, it was still possible to understand the breeding system of the plant. *Rhodophiala* did not set seeds autogamously. The stigmas seemed to be receptive at anthesis because the stigmas collected to examine pollen tube formation by cross pollination and self-pollination all showed pollen tube growth. The mean herkogamy distance was 10.5 ± 0.6 mm ($n = 30$), and it is very unlikely that the pollen could move from the anthers to the stigma unassisted in an

unvisited flower. The plant was partially self-compatible (mixed mating system), but fruit and seed sets for the selfed treatments were significantly lower than those in the cross or open pollination treatments (Fig. 3). Although the supplemental pollination increased the fruit set, it slightly decreased the seed set, but not significantly. The seed and fruit sets under open pollination were almost identical between sites 1 and 2, although only 14 flowers were recovered from site 2. The natural seed production was 10–15-fold that of the estimated current flowering plant density. Only three flowers (out of 15) in the 'clog' treatment escaped predation by animals. Only two out of these three flowers produced a fruit and the proportion of ovules producing a seed was low (0.18), commensurate with the selfing treatment.

The estimated range of seed production for site 1 calculated from the 95% confidence intervals of reproductive plant density was 1.9–5.4 seeds/m^2, considerably higher than the present estimated plant density (Table 1).

Discussion

Despite very different floral and vegetative morphologies, these two alpine species have considerable similarities in their breeding systems and reproductive output.

Nastanthus has a number of features related to wind pollination, such as a long and exserted stigma, greenish flowers, dry pollen and no nectar; however, the likelihood of wind pollination was discarded. Pollen is presented completely covering the stigma, which is different from many flowers with a pollen presenter (Ladd 1994). In addition, the compact

growth form would not be conducive to abiotic pollen removal. Thus, the pollen needs to be removed by animal vectors, although no insect visitors were observed in our study. As the *Nastanthus* flowers offer only pollen, tiny Andrenid bees found in the high central Chilean Andes (e.g. Arroyo *et al.* 2006), which collect pollen for brood provisioning, might visit the flowers. These extremely rapidly moving bees tend to be active only under warm and windless conditions. Nevertheless, the proportion of flowers producing fruits was high. This is consistent with the monocarpic habit of this species and a population structure with a preponderance of small non-reproductive plants that will eventually become reproductive.

Rhodophiala, in contrast to *Nastanthus*, has large and colorful flowers. Its herkogamous features indicate the necessity of animal pollinators. The main flower visitor observed harvesting pollen, *Megachile sauleyi*, is of a suitable size and carries the pollen under its abdomen in just the correct place to contact the stigma if the bee approaches the flower in line with the style. In a strict sense, the plant has a mixed mating system because some of the seeds were set by selfing, but this seed set was significantly lower than that by outcrossing. Bulbous species tend to be self-incompatible and the fruit set in *Rhodophiala* was similar to that in the amaryllid *Hippeastrum adventum* for both self-pollination and cross pollination (Saavedra *et al.* 1996). Self-pollen deposition on stigmas preceding cross-pollen deposition might be detrimental to maximal seed set. Pollen delivery does not seem to limit the seed set in contrast to what is often claimed for alpine areas (Molau 1993; Blionis & Vokou 2005; Raffl *et al.* 2007).

How do these species accord with some of the models proposed for pollination and seed set in alpine zones? It has been suggested that the harsh alpine environment might lead to the evolution of wind pollination or selfing (Billings 1974; Anderson 2000). However, Arroyo *et al.* (1985) pointed out that alpine flowers often have greater longevities than flowers at lower elevations and that this property compensates for low pollinator activity (see also Bingham & Orthner 1998; Fabbro & Körner 2004). In fact, many species are self-incompatible in the Patagonian alpine (Arroyo & Squeo 1990). Fabbro and Körner (2004) provide evidence of proportionately higher biomass in the flowers of alpine plants compared with vegetative organs and state that high genetic variability in a number of alpine species indicates 'considerable outcrossing'.

Extended flower longevity and large displays are commensurate with the views of other authors that alpine plants invest heavily in reproduction. For *Nastanthus*, the plant body at full anthesis is completely covered with flowers and virtually no foliage parts are visible. Unlike species, such as *Androsace alpina* and *Silene acaulis* (Fabbro & Körner 2004), however, self-shading by the reproductive parts is unlikely to be problematic because the flowers are green and are likely to contribute to their own seed provisioning. The plants also have a large fleshy tap-root system and all of the stored resources can be used for seed production because the plant dies after reproduction. Similarly, in *Rhodophiala* the fruits should be provisioned from resources stored in the bulb because no leaves remain at anthesis. The seed set in pollinated flowers was relatively high compared with that in some other species in alpine environments, for example, *Chuquiraga oppositifolia* (Muñoz & Arroyo 2006), *Astragalus alpinus* (Kudo & Molau 1999) and *Saxifraga oppositifolia* (Gugerli 1998). Pollen : ovule ratios of both species were large and within the range of facultative

xenogamy/xenogamy (*Nastanthus*) and complete xenogamy (*Rhodophiala*) given by Cruden (1977) and also commensurate with the generally higher pollen : ovule ratios of other species that provide pollen rewards to visitors (cf. Cruden 2000).

Attention from pollinators appears to have been adequate, at least in the year of the present study, which was an unusually dry (La Niñã) year. The flower visitation rate to *Rhodophiala* (0.00045 visits/h/flower) was fourfold higher than that recorded for the small, highly autogamous alpine daisy (*Chaetanthera euphrasioides*, 0.00011 visits/h/flower) in the same area, but at a higher altitude (Arroyo *et al.* 2006). However, the rate was much less than the visitation rates recorded for *Ranunculus* (2.76 visits/h/flower) at an altitude of 1490 m in Norway (Totland 2001) and 0.5 visits/h to *Dryas integrifolia* in Greenland (Philipp *et al.* 1990). Nevertheless, it might not be realistic to compare visitation rates among geographical regions, on account of local differences in weather conditions. *Megachile* spends only 1–2 s in each flower, but might make repeated visits to the same flower, and also flies erratically from one area to another. Thus, many flowers can be visited in a short time.

In *Nastanthus,* although no flower visitors were recorded in our study, the fruit set was very high. This species is more strongly self-incompatible than *R. rhodolirion*, and self-pollen would be removed from the stigma automatically if cross pollen was delivered by a flower visitor. This is similar to a related genus *Campanula* that also has a pollen presenter. A study of nine species on an altitudinal gradient on Mt Olympos in Greece showed that all species were non-autogamous and that the two species that encompassed the entire range were also self-incompatible (Blionis & Vokou 2005). At present, the seed production of both species

examined in the present study is most strongly influenced not by intrinsic attributes of the species or the natural environment, but by grazing/predation by introduced free ranging cattle and horses. This is similar to the situation on Mt Olympos in the true Mediterranean, where goats and sheep graze the alpine pastures in summer and remove a considerable proportion of the seed production of *Campanula* and presumably other taxa (Blionis & Vokou 2005).

Given the various compensatory mechanisms that might be acquired by alpine plants to compensate for low flower visitation rates, Arroyo *et al.* (2006) predicted a diversity of breeding systems in alpine areas. Although few species have been studied to date, this certainly appears to be the case for the Mediterranean alpine site in central Chile examined in the present study. Published breeding system records at exactly the same site are available for 10 other species. Including the new information obtained for *N. spathulatus* and *R. rhodolirion* in our study, breeding systems among the nine hermaphroditic species range from a very high level of autogamy (*Chaetanthera euphrasioides*, Asteraceae; Arroyo *et al.* 2006) with no pollen limitation, to mixed mating in showy, well-visited flowers (*Anarthrophyllum cumingii*, Fabaceae; Rozzi *et al.* 1997; *Chaetanthera apiculata* and *Chaetanthera lycopodioides*; Torres-Díaz *et al.* 2007); *R. rhodolirion* with no detectable pollen limitation in the case of the last species, to self-incompatibility with no detectable pollen limitation (*N. spathulatus*), self-incompatibility with significant pollen limitation (*Chuquiraga oppositifolia*, Asteraceae; Muñoz & Arroyo 2006), heteromorphic incompatibility associated with tristyly and considerable pollination limitation (*Oxalis squamata*, Oxalidaceae; Marco & Arroyo 1998) and gynodioecy with some pollen limitation on both female and hermaphrodite plants (*Nototriche compacta*, Malvaceae; García-Franco & Arroyo 1995). Dioecy is also represented (e.g. *Antennaria chilensis*, Asteraceae; *Laretia*

acaulis, Apiaceae; *Ribes cucullatum*, Grossulariaceae). This preliminary sample of 12 species of a very large and taxonomically diverse alpine flora not only shows that almost all known breeding systems in the angiosperms coexist at a single site, but suggests that obligate outbreeding or mixed mating will turn out to be frequent in the Mediterranean alpine of central Chile.

The contrast between the pattern of breeding systems of tundra species (Molau 1993) and those of the Chilean alpine might be related to the evolutionary source of the taxa for the two areas. Tundra species have presumably had a long history of evolution under conditions of a short growing season and harsh climatic conditions and floras would have moved in relation to the advancing and retreating ice sheets through the Quaternary, but always under a short growing season. In contrast, the source for many Chilean alpine taxa is the lowland Mediterranean-type climate flora of Chile. Taxa such as *Rhodophiala* range from the coast to alpine areas. As noted by Arroyo *et al.* (2006), in relation to *Chaetanthera euphrasioides*, the breeding systems of alpine species are often similar to their lowland relations and the alpine species have presumably inherited these systems from their ancestors and taken them to the alpine zone.

Acknowledgments

References

Anderson G. J. (2000) Dioecy and wind pollination in *Pernettya rigida* (Ericaceae) of the Juan Fernandez Islands. *Botanical Journal of the Linnean Society* **132**: 121–141.

Arroyo M. T. K. & Squeo F. A. (1987) Experimental detection of anemophily in *Pernettya mucronata* (Ericaceae) in western Patagonia, Chile. *Botanische Jahrbucher Systematic* **108**: 537–546.

Arroyo M. T. K. & Squeo F. A. (1990) Relationships between plant breeding systems and pollination. In: Kawano S. (ed.). *Biological Approaches and Evolutionary Trends in Plants*. Academic Toronto Press, London, pp. 205–227.

Arroyo M. T. K., Primack R. B. & Armesto J. J. (1982) Community studies in pollination ecology in the high temperate Andes of central Chile I. Pollination mechanisms and altitudinal variation. *American Journal of Botany* **69**: 82–97.

Arroyo M. T. K., Armesto J. J. & Primack R. B. (1985) Community studies in pollination ecology in the high temperate Andes of central Chile II. Effect of temperature on visitation rates and pollination possibilities. *Plant Systematics and Evolution* **149**: 187–203.

Arroyo M. T. K., Munoz M. S., Henriquez C., Till-Bottraud I. & Perez F. (2006) Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above tree-line species in the high Andes of Chile. *Acta Oecologica* **30**: 248–257.

Bergman P., Molau U. & Holmgren B. (1996) Micrometeorological impacts of insect activity and plant reproductive success in an alpine environment. *Arctic and Alpine Research* **28**: 196–202.

Billings W. D. (1974) Arctic and alpine vegetation: plant adaptations to cold summer climates. In: Ives J. D. & Barry R. G. (eds). *Arctic and Alpine Environments*. Methuen, London, pp. 403–438.

Bingham R. A. & Orthner A. R. (1998) Efficient pollination of alpine plants. *Nature* **391**: 238–239.

Blionis G. J. & Vokou D. (2005) Reproductive attributes of *Campanula* populations from Mt Olympos, Greece. *Plant Ecology* **178**: 77–88.

Bremer K. & Gustafsson M. H. G. (1997) East Gondwana ancestry of the sunflower alliance of families. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 9188–9190.

Cruden R. W. (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31**: 32–46.

Cruden R. W. (2000) Pollen grains: why so many? *Plant Systematics and Evolution* **222**: 143–165.

Fabbro T. & Körner C. (2004) Altitudinal differences in flower traits and reproductive allocation. *Flora* **199**: 70–81.

García-Franco J. G. & Arroyo M. T. K. (1995) Breeding system, sex ratio and individual size of the gynodioecious *Nototriche compacta* (Malvaceae) in the Andes of Central Chile. *Plant Species Biology* **10**: 147–153.

Gugerli F. (1998) Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia* **114**: 60–66.

Hoffmann A., Arroyo M. K., Liberona F., Muñoz M. & Watson J. (1998) *Plantas Altoandinas en la flora Silvestre de Chile*. Imprenta Salesianos, Santiago.

Krebs C. J. (1989) *Ecological Methodology*. Harper & Row, New York.

Kudo G. & Molau U. (1999) Variation in reproductive traits at inflorescence and flower levels of an arctic legume, *Astragalus alpinus* L.: comparison between a subalpine and an alpine population. *Plant Species Biology* **14**: 181–191.

Körner C. (1999) *Alpine Plant Life*. Springer, Berlin.

Ladd P. G. (1994) Pollen presenters in the flowering plants: form and function. *Botanical Journal of the Linnean Society* **115**: 165–195.

Linder H. P. (1998) Morphology and evolution of wind pollination. In: Owens S. J. & Rudall P. J. (eds). *Reproductive Biology*. Royal Botanic Gardens, Kew, pp. 123–135.

Marco D. E. & Arroyo M. T. K. (1998) The breeding system of *Oxalis squamata*, a tristylous South American species. *Botanica Acta (Germany)* **111**: 497–504.

Molau U. (1993) Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research* **25**: 391–402.

Mosquin T. (1966) Reproductive specialization as a factor in the evolution of the Canadian flora. In: Taylor R. L. & Ludwig R. A. (eds). *The Evolution of Canada's Flora*. University of Toronto Press, Toronto, pp. 43–65.

Muñoz A. A. & Arroyo M. T. K. (2006) Pollen limitation and spatial variation of reproductive success in the insect pollinated shrub *Chuquiraga oppositifolia* (Asteraceae) in the Chilean Andes. *Arctic, Antarctic and Alpine Research* **38**: 608– 613.

Philipp M., Böcher J., Mattsson O. & Woodell S. R. J. (1990) A quantitative approach to the sexual reproductive biology in some arctic flowering plants: *Dryas integrifolia*, *Silene acaulis* and *Ranunculus rivularis*. *Meddeleser om Grønland, Bioscience* **34**: 1–60.

Raffl C., Marcante S. & Erschbamer B. (2007) The role of spontaneous selfing in the pioneer species *Saxifraga aizoides*. *Flora* **202**: 128–132.

Rathke B. J. (2003) Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *American Journal of Botany* **90**: 1328–1332.

Rozzi R., Arroyo M. T. K. & Armesto J. J. (1997) Ecological factors affecting gene flow between populations of *Anarthrophyllum cumingii* (Papilionaceae) growing on equatorial and polar-facing slopes in the Andes of Central Chile. *Plant Ecology* **132**: 171–179.

Saavedra F., Arroyo M. T. K. & Hoffman A. J. (1996) The breeding system of *Hippeastrum adventum* (Ker-Gwal.) Herb. (Amaryllidaceae), an endemic bulbous geophyte of the mediterranean-type climate region in central Chile. *Botanische Jahrbücher Systematik* **118**: 1–8.

Scheffknecht S., Dullinger S., Grabherr G. & Hulber K. (2007) Mating systems of snowbed plant species of the north-eastern calcareous alps of Austria. *Acta Oecologica* **31**: 203–209.

Shimono A. & Washitani I. (2007) Factors affecting seed production in the heterostylous herb *Primula modesta*. *Plant Species Biology* **22**: 65–76.

Torres-Díaz C., Cavieres L. A., Muñoz-Ramírez C. & ArroyoM. T. K. (2007) Consecuencias de las variaciones microclimáticas sobre la visita de insectos polinizadores en dos especies de *Chaetanthera* (Asteraceae) en los Andes de Chile central. *Revista Chilena de Historia Natural* **80**: 455–468.

Totland Ø. (1994) Influence of climate, time of day and season, and flower density on insect visitation in alpine Norway. *Arctic and Alpine Research* **26**: 66–71.

Totland Ø. (2001) Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology* **82**: 2233–2244.

Figure 1. *Nastanthus spathulatus.*

Figure 2. *Rhodophiala rhodolirion.*

Figure 3. Fruit set and mean seed set for *Rhodophiala rhodolirion* in the different pollination treatments at the La Parva site (site 1). Seed set bars with different letters are significantly different (Mann–Whitney *U*-test; $P < 0.05$). Bars indicate standard error.
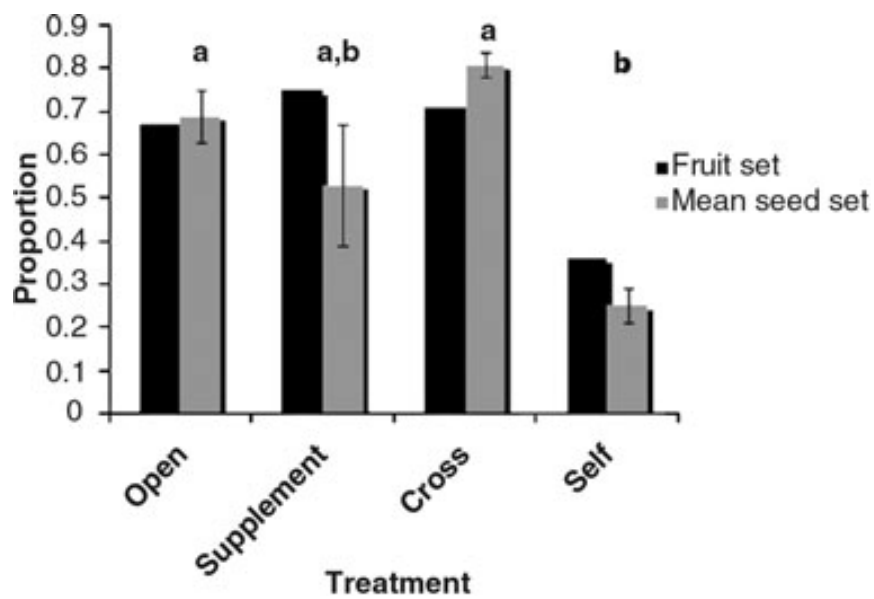
Table 1. Morphological and reproductive characteristics of *Nastanthus spathulatus* and *Rhodophiala rhodolirion* flowers

	Nastanthus	*Rhodophiala*
Inflorescence	Capitulum	Solitary Flower
color	Greenish	White to pink
Nectar	None	None
Flower odor	None	None
Pollen presented on stigma	Yes	No
Pollen grains per flower	868 ± 88 ($n = 6$)	$294\ 251 \pm 66\ 291$ ($n = 6$)
Ovules per flower	1	45.4 ± 2.1 ($n = 20$)
Pollen : ovule ratio	868	6481
Mean plant density per m^2 (95% confidence intervals)	0.067 (0.045–0.135)	Site 1: 0.135 (0.099–0.209)
		Site 2: 0.131 (0.088–0.255)
Proportion of plants fertile	0.11	1.0
Inflorescences number per plant	24.8 ± 8.8 ($n = 5$)	na
Estimated seed production (seeds per m^2)	6.5–19.5	1.9–5.4

Mean $\pm$ standard error. na, not applicable.

Table 2. Results of the seed set and pollen tube analysis of *Nastanthus* flowers in the pollination trials and in the broad-scale open pollination

Treatment	Proportion of fruits with seed	Proportion of stigmas with pollen tubes
Open pollination	0.76 ± 0.08 ($n = 4$)	0.71 ($n = 2$)
Open pollination with supplemental cross pollen	0.86 ± 0.08 ($n = 3$)	0.76 ± 0.09 ($n = 4$)
Autogamy	0.02 ± 0.01 ($n = 9$)	0 ($n = 6$)
Population seed set La Parva	0.92 ± 0.01 ($n = 36$)	na
Population seed set Portillo	0.67 ± 0.10 (n = 8)	na

Mean ± standard error. na, not measure