Pollination of plants is crucial for reproductive success, and understanding the factors that limit pollination can provide insights into plant adaptation and biodiversity. Insect-pollinated plants, in particular, have evolved various strategies to overcome the challenges posed by pollinator availability and activity. In the alpine environment, where temperatures are low, growing seasons are short, and wind speeds are high, pollinator visitation can be limited, potentially leading to pollen limitation (PL). PL is a phenomenon where pollinator visitation is insufficient to meet the reproductive demands of the plant, thereby reducing reproductive success.

**Abstract**

Low temperatures, short growing seasons, and strong winds, which constrain the abundance and activity of insect pollinators, characterize alpine ecosystems. In northern hemisphere alpine environments, the reproductive output of several insect-pollinated plants has been reported to be pollen-limited. Using a supplemental hand-pollination experiment, we assessed the magnitude of pollen limitation (PL) in the obligate outcrossing insect-pollinated shrub *Chuquiraga oppositifolia* in the lower alpine scrub vegetation belt in the central Chilean Andes. We also assessed spatial variation in its reproductive success by comparing seed production among three additional sites. Hand-pollination resulted in a two- to three-fold increase in seed output above natural levels, thus demonstrating PL in this species. Nevertheless, percentage seed output remained low, increasing from 2.0 to 5.7%. Seed weight was reduced by 15% in hand-pollinated plants. Seed output was also low in the three additional sites, but did not differ among them, suggesting that low seed output is a spatially widespread phenomenon in this species. Our results, together with previous research, suggest that both pollinator visitation and abiotic resources (soil nutrients) constrain the reproductive output of this shrub. Longer-term research should unravel potential future vegetative and reproductive costs of current-year enhanced reproductive output and determine if PL is a recurrent phenomenon in the Chilean Andes.

**Introduction**

In flowering plants, much research has shown seed and fruit output to be constrained by insufficient pollen delivery to stigmas, a phenomenon known as “pollen limitation” (hereafter “PL”). In the southern hemisphere, this phenomenon is well documented in alpine ecosystems (e.g., Galen, 1985; Whelan and Goldingay, 1989; Burd, 1994; Larson and Barrett, 2000; Ashman et al., 2004). At the whole plant level, PL is demonstrated empirically when supplemental hand pollination of flowers increases seed output of individuals compared to open pollinated controls. In a literature review, Burd (1994) reported significant PL at some sites and in some sites in 62% of 258 species examined. More recently, Larson and Barrett (2000) documented PL to be ca. twice as intense in obligate outcrossing species than in self-compatible and autogamous species, which can be explained by the dependence of outcrossers on variable and unpredictable insect pollinator visitation.

Due to the low ambient temperatures, short snow-free growing seasons, and strong winds that high-mountain or alpine areas experience, a phenomenon known as “pollen limitation” is often critical for reproductive success. PL is demonstrated empirically when supplemental hand pollination of flowers increases seed output of individuals compared to open pollinated controls. In a literature review, Burd (1994) reported significant PL at some sites and in some sites in 62% of 258 species examined. More recently, Larson and Barrett (2000) documented PL to be ca. twice as intense in obligate outcrossing species than in self-compatible and autogamous species, which can be explained by the dependence of outcrossers on variable and unpredictable insect pollinator visitation.
in a strong increase in its female reproductive success, thus providing evidence for PL. In addition, we assessed whether there is spatial variation in its reproductive success. To address this question, we compared seed production of _C. oppositifolia_ among three different sites within the subbande scrub vegetation belt in the central Chilean Andes. Given the frequently low abundance and activity levels of insect pollinators reported for this alpine ecosystem (Arroyo et al., 1982, 1983; Rozzi, 1990; Muñoz and Arroyo, 2004), we predicted that seed output would be low at all three sites. Such a result would be suggestive of pollen limitation being a spatially widespread phenomenon in the central Chilean Andes. Thus, we asked the following questions: (1) Is seed production in this alpine shrub limited by pollen availability? and (2) Does its female reproductive output vary spatially within the lower alpine scrub vegetation belt?

**Methods**

**THE PLANT SPECIES**

The dwarf <45-cm-high spiny shrub _Chuquiraga oppositifolia_ (Asteraceae: Mutisieae) is the dominant shrub species at many sites within the subbande scrub vegetation belt (2000–2700 m elevation) in the central Chilean Andes (Cavieres et al., 2000; A.A.M., personal observations). It flowers late in the growing season (January to April) (Arroyo et al., 1981) and becomes replete with large 10- to 12-mm-diameter, golden-yellow capitula containing 9 to 12 morphologically identical disc florets on average (Rozzi, 1990; Muñoz, 2003), which are insect-pollinated (Arroyo et al., 1982; Rozzi, 1990). In this species, all florets are fertile and each is capable of developing a single seed. Percentage seed set within each capitulum ranges widely from 0 to 100% (Muñoz, 2003) although mean number of achenes produced per capitulum is <1 (Muñoz and Arroyo, 2004). A breeding system analysis using bagging starting at the bud stage and controlled self- and cross-pollinations showed that _C. oppositifolia_ requires visitation to produce seeds (Rozzi, 1990; Rozzi, unpublished data). Bagged capitula, which insects could not access, on 15 individual shrubs, did not produce any seeds, thus showing that _C. oppositifolia_ is not autogamous (or capable of self-fertilization). Open pollination by insects resulted in seed set, whereas bagging only did so following cross-pollination, thus demonstrating that it is fully self-incompatible and hence outcrossing (Rozzi, 1990; Rozzi, unpublished data). The most important pollinators of _C. oppositifolia_ are the satyrid butterfly _Cosmosatyrus chilensis_, the syrphid fly _Scaeva melanostoma_, and the andrenid bee _Heterosaurus sp._ although it is also visited by many other taxa including bombylid and tachinid flies, other small andrenid bees, and the bumblebee _Bombus dahliibomii_ (Muñoz, 2003; Muñoz and Arroyo, 2004; see also Arroyo et al., 1982). Detailed pollen-load analyses demonstrated that the main pollinators of _C. oppositifolia_ carry abundant pollen (Rozzi, 1990).

**STUDY SITES**

Research was conducted at four sites within the subbande scrub vegetation belt in the central Chilean Andes (32°–33°S). The supplemental hand-pollination experiment was performed at an 18-ha site at 2600 m elevation near the Valle Nevado (VN) Ski Resort (33°21’S, 70°16’W) between January and May 2002. Here, climate is alpine with Mediterranean influence, with a 5- to 8-mo snow-free growing season, commonly extending from mid-October to mid-May (Arroyo et al., 1981). This study site is south-facing with gentle (<15°) slopes. Vegetation is dominated by low (<45 cm) spiny shrubs of _Chuquiraga oppositifolia_ (Asteraceae), _Anarthrophyllum cumingii_ (Papilionaceae), and _Berberis empetrifolia_ (Berberidaceae). Herbaceous species, such as _Acaena pinnatifida_ (Rosaceae), _Phacelia secunda_ (Hydrophyllaceae), _Stachys philippiana_ (Labiatae), and various species of _Adesmia_ (Papilionaceae) and _Senecio_ (Asteraceae), are also abundant.

To evaluate whether low seed production in _Chuquiraga oppositifolia_ is a spatially widespread phenomenon in the central Chilean Andes, three additional study sites were selected in December 2002. These sites were: Lagunillas (LA) at 2000 m a.s.l. (33°33’S, 70°16’W) and ca. 30 km south of VN, Farellones (FA) at 2300 m a.s.l. (33°19’S, 70°17’W) and ca. 5 km west of VN, and Portillo (PO) at 2550 m a.s.l. (32°51’S, 70°10’W) and ca. 60 km north of VN. LA contains a mixture of subbande scrub vegetation dominated by _C. oppositifolia_, _Acaena pinnatifida_, _Mutisia sinuata_ (Asteraceae), and various species of _Senecio_ and _Adesmia_, together with vegetation characteristic of the montane sclerophyllous matorral immediately below it, dominated by the trees _Kageneckia angustifolia_ (Rosaceae) and _Schinus montanus_ (Anacardiaceae), and the shrub _Colliguaja integerrima_ (Euphorbiaceae). This study site is west-facing with gentle (<20°) slopes. Vegetation at FA is very similar to that described for the VN site, with gentle (<15°) slopes but has a west-facing aspect. Vegetation at PO is dominated again by _C. oppositifolia_ and _B. empetrifolia_. The other dominant species here are _Tropaeolum polyphyllum_ (Tropaeoleaceae) and _Lathyrus sp._ (Papilionaceae). This site is south-facing with steep (20–50°) slopes.

**POLLEN LIMITATION EXPERIMENT**

We employed supplemental hand-pollination to assess whether the amounts of pollen reaching stigmas constrain seed production in _Chuquiraga oppositifolia_. In January 2002, in a fairly flat 200 × 75 m (1.5 ha) sector within the 18-ha site at VN, we randomly selected a total of 30 similarly sized (ca. 0.2–0.3 m ³) shrubs. Selected experimental shrubs were located at least 5 m away from each other so as to study clearly distinct individual shrubs, thus ensuring independence of replicates. All shrubs studied possessed 350 to 500 apical floral buds at this stage, being representative of individuals growing in the general area in terms of size and floral display. Each shrub was randomly assigned to either a supplemental pollination (n = 15 shrubs) or control (n = 15 shrubs) treatment. On each individual we tagged 50 randomly selected twiglets, each with its apical floral bud as in Muñoz et al. (2005). Each twiglet can produce only one capitulum. Given that this species is self-incompatible (see above), we collected the pollen used to hand pollinate the 15 plants under the supplemental pollen treatment from shrubs that were growing at least 50 m away from the 1.5-ha experimental sector of the study site. A 50-m distance allowed us to avoid potentially disturbing nontarget control plants within the experimental sector. At the same time, this distance was well within the flying range of the main insect pollinators of this species including the butterfly _Cosmosatyrus chilensis_, the fly _Scaeva melanostoma_, and the bumblebee _Bombus dahliibomii_ (personal observations). Thus, natural cross-pollination could also be expected between donor and target plants. To accomplish pollen addition, we collected a large quantity of florets with dark orange pollen-laden anthers from the above plants and carefully brushed these across the receptive stigmas of florets of most (>80%) of the total number of capitula on the supplemental pollination plants (including all 50 capitula that were tagged). Thus, our experimental procedure allowed us to test for PL at the
whole-plant level (Zimmerman and Pyke, 1988). We hand-pollinated shrubs on three occasions during peak flowering of each individual between late January and late February 2002.

We covered all monitored capitula following withering in late February–early March with 8×6 cm yellow mesh bags so as to prevent the potential loss of the developing wind-dispersed achenes (one-seeded fruit) as in Muñoz and Arroyo (2004). We retrieved all bags in April–May (thus allowing sufficient time for seed development), and analyzed each capitulum for seed output (number of achenes) and seed quality (weight of achenes). Seed output was expressed as (1) percentage of capitula per shrub that set one or more achenes (%CA), (2) mean number of achenes per capitulum per shrub (NAC), and (3) percentage seed set (%SS), which was measured as the percentage of ovaries of open florets per shrub that set seeds (i.e., that could potentially have been fertilized). Percentage seed set (%SS) is equivalent to the seed:ovule (s:o) ratio reported in other studies (e.g., Gugerli, 1998; Totland, 2004). When calculating NAC, we included all monitored capitula regardless of whether the capitulum produced any achenes or not (i.e. including the zero-achene capitula). Additionally, we determined the frequency distribution of seed output per capitulum (i.e., the percentage of capitula with 0, 1, 2, 3, >3 achenes) for control and supplemental hand pollination shrubs. Then, we individually weighed dry achenes (including the pappus) to the nearest milligram using a digital balance. We analyzed differences in seed output and quality in control vs. supplemental pollination shrubs using Mann-Whitney U tests because the assumption of normality of data for parametric tests was not met, even when appropriately transformed (Zar, 1996).

**Results**

**POLLEN LIMITATION EXPERIMENT**

Supplemental hand-pollination resulted in a significant enhancement in seed output of *C. oppositifolia* (Fig. 1). Mean percentage of capitula per shrub that set one or more achenes (%CA) was more than two-fold higher in hand-pollinated shrubs compared to control shrubs (Mann-Whitney U test, *U* = 24.0, *z* = −3.65, *P* < 0.001, Fig. 1a). Likewise, hand-pollinated shrubs produced a > three-fold greater number of achenes per capitulum per shrub (NAC) than control shrubs (*U* = 23.5, *z* = −3.65, *P* < 0.001, Fig. 1b). Percentage seed set (%SS) was also significantly lower in control compared to hand-pollinated shrubs (*U* = 24.5, *z* = −3.65, *P* < 0.001, Fig. 1c). Additionally, almost three times as
many capitula from supplemental hand pollination shrubs (34.05%) produced 1 to 3 achenes compared to capitula on control shrubs (12.27%). However, seed quality, expressed as the mean achene mass per shrub, was slightly reduced in hand-pollinated vs. control shrubs ($U = 64.0$, $z = 2.012$, $P = 0.044$, Fig. 1d).

**SPATIAL PATTERNS OF SEED PRODUCTION IN THE SUBANDEAN SCRUB BELT**

Seed output in shrubs of *C. oppositifolia* did not differ among the three sites studied when expressed as %CA ($F_{1,27} = 1.019$, $P = 0.374$), NAC ($F_{1,27} = 1.368$, $P = 0.272$), and %SS ($F_{1,27} = 1.542$, $P = 0.232$) (Fig. 2 a–c). However, mean weight of achenes produced by shrubs at the FA and PO sites was ca. 20 and 30% lower, respectively, than that of achenes produced at the LA site (Fig. 2d, $F_{1,27} = 16.056$, $P < 0.01$, a posteriori Tukey HSD, LA $\neq$ FA = PO, $P < 0.05$).

On the other hand, control (natural) seed output at the experimental site at Valle Nevado, was significantly lower compared to all three other sites, expressed as %CA ($F_{1,41} = 9.388$, $P < 0.01$, a posteriori Tukey HSD, $P < 0.05$), NAC ($F_{1,41} = 7.871$, $P < 0.05$, a posteriori Tukey HSD, $P < 0.05$), and %SS ($F_{1,41} = 8.918$, $P < 0.01$, a posteriori Tukey HSD, $P < 0.05$) (Figs. 1, 2).

**Discussion**

Female reproductive success of the fully self-incompatible insect-pollinated dwarf shrub *Chuquiraga oppositifolia* was strongly limited by pollen availability at the Valle Nevado site, with seed production of naturally open pollinated shrubs being very low there. Supplemental hand pollination at the individual shrub level resulted in a two- to three-fold increase in seed output above natural levels at the Valle Nevado site. This result concurs with reviews documenting that long-lived, woody, and self-incompatible species are often pollinated limited (Burd, 1994; Larson and Barrett, 2000). As far as we are aware, our study is the first to directly test for, and document, PL in an alpine species in a southern hemisphere high-altitude ecosystem in general, and in the central Chilean Andes in particular. Additionally, seed output was also low and did not differ among the three additional sites examined (Lagunillas, Farellones, and Portillo) within the subande scrub vegetation belt, suggesting that low seed output is a spatially widespread phenomenon in this shrub species within this ecosystem. Nevertheless, it is worth pointing out that seed output at these three sites was higher than at Valle Nevado, with supplemental hand pollination there having brought seed production into the range of natural reproductive output at Lagunillas, Farellones, and Portillo.

In other studies in alpine ecosystems, a lack of PL of the reproductive success of some species or populations has sometimes been reported (e.g., Young, 1982; Sandvik et al., 1999; Totland, 2004). This has often been attributed to the possession of mixed (e.g., insect and wind) pollination strategies (Totland and Sottocornola, 2001), or the capacity for self-pollination (autogamy) (Young, 1982; Kasagi and Kudo, 2003) and self-fertilization (self-compatibility) (Sandvik et al., 1999; Totland and Schulte-Herbrüggen, 2003). Such species are not fully dependent on cross-pollination by insects to produce seeds as in *C. oppositifolia*.

Strong PL was expected in *C. oppositifolia* in view of its reliance on cross-pollination for seed production (Rozzi, 1990) and the already reported low pollinator visitation rates to this shrub species in previous field experiments (Muñoz and Arroyo, 2004; Muñoz et al., 2005). Indeed, the occurrence of PL at a number of northern hemisphere high-mountain ecosystems, including the Rocky Mountains of North America (Galen, 1985; Campbell and Halama, 1993), alpine southwest Norway (e.g., Totland, 2001; Totland and Sottocornola, 2001) and the Taishetsu Mountains of northern Japan (Kudo and Suzuki, 2002; Kasagi and Kudo, 2003) could be explained by the low diversity, abundance, and activity levels of insect pollinators in cold-weather alpine ecosystems (e.g., Arroyo et al., 1982, 1985; Galen, 1985; Kearns, 1992; McCall and Primack, 1992; Kudo, 1993; Totland, 1994a, 1994b; Bingham and Orthner, 1998; Medan et al., 2002; Muñoz and Arroyo, 2004). However, Totland (2001) cautioned that low pollinator availability or visitation at high altitudes does not always lead to strong PL of seed output, even in self-
incompatible species. Seed set of the perennial herb *Ranunculus acris* in a high-altitude population in alpine southwest Norway was not enhanced via supplemental hand-pollination despite naturally low pollinator visitation rates, presumably because seed production there was constrained by resource availability, particularly by low temperatures (Totland, 2001, 2004). In contrast, seed output in a lower-altitude population, where pollinator visitation rates were three times higher compared to the high-altitude population, was pollen limited (Totland, 2001).

In our study, despite a three-fold enhancement in seed set of *C. oppositifolia* following supplemental hand-pollination, seed set still remained very low, reaching only 5.7% (despite that capitula are capable of maturing all seeds produced). This result, along with the recent demonstration that seed output in *C. oppositifolia* is also strongly limited by soil nitrogen resource availability (Muñoz et al., 2005), reinforces the notion that plant reproductive success in alpine ecosystems can be constrained by both resources and pollinators (Haig and Westoby, 1988; Campbell and Halama, 1993; Totland, 2004). In our nitrogen addition study (Muñoz et al., 2005), we argued that the ca. three- to four-fold enhancement in seed output in shrubs that had received nitrogen fertilizer was probably a result, not only of the greater amount of resources available for seed maturation, but also of the ca. two-fold enhancement in floral display, ending in a ca. two-fold increase in pollinator visitation, and ultimately in the liberation of these shrubs from PL.

Alternatively, the observed low seed output in this self-incompatible alpine shrub (both naturally at the four sites and when hand pollinated) may be caused by natural selection for large floral displays in this system, (where pollinator abundance and activity levels are low), resulting in the production of many more capitula and florets than would be needed to achieve full seed set. Large floral displays would enhance male fitness (siring success) through pollen removal (e.g., Mutikainen and Delph, 1996; Strauss, 1997; Krupnick and Wess, 1999). Here, we assessed the reproductive success of *C. oppositifolia* through the female function only (i.e., seed output).

Additionally, a particularly that may be relevant in the *C. oppositifolia*-insect pollinator system is that this abundant, annually flowering, and long-lived woody plant, through its often large floral displays, may be maintaining the populations of pollinators via its food “offerings.” This type of plant-pollinator mutualism, suggested for in other systems (e.g., Scott et al., 1993), may be important in the central Chilean Andes since it is known that, on the one hand, a high percentage of species are insect-pollinated (Arroyo et al., 1982; Muñoz, unpublished data) and, on the other, visitation rates are very low (Arroyo et al., 1985; Muñoz and Arroyo, 2004).

Nevertheless, because our study was conducted during a single growing season only, we cannot assert that the reproductive output of *C. oppositifolia* necessarily would be pollen limited during subsequent seasons. Relatively few studies have examined PL over multiple growing seasons (see Burd, 1994; and Larson and Barrett, 2000, for reviews), and, to our knowledge, only two recent studies (Kasagi and Kudo, 2003; Totland, 2004) have done so for high-altitude plant species. In other environments, PL has been shown to vary seasonally (e.g., Ramsey, 1995; Baker et al., 2000; Goldingay, 2000), with interyear shifts in pollinator abundance and activity being invoked to explain such changes in PL (e.g., Ramsey, 1995). In the Andes Mountains of central Chile, the recently documented extremely low insect pollinator visitation rates to *C. oppositifolia* during two growing seasons (2001–2002 and 2003–2004) at the VN study site (Muñoz and Arroyo, 2004; Muñoz et al., 2005), together with the low visitation rates at nearby sites during the 1984–1985 growing season (Rozzi, 1990), are suggestive that seed output in *C. oppositifolia* is often pollen limited. Interannual variation in seed output can also be due to costs of reproduction. Some experimentally hand-pollinated species showed reduced vegetative growth or reproduction in subsequent years (e.g., Ackerman and Montalvo, 1990; Primack and Hall, 1990; Mattila and Kuitunen, 2000), although such costs have not been seen in others (e.g., Ramsey, 1995; Goldingay, 2000). Thus, through elevated seed output in one season, some plants may experience a reduction in lifetime fitness. Although we currently lack data on future potential costs of enhanced seed output in hand-pollinated shrubs, our results showing an immediate (current season) reduction in the mass of achenes produced by supplemental pollinated shrubs suggest that such costs occur in *C. oppositifolia*.

To attempt to further understand the relative importance of pollen vs. resource limitation towards the longer-term reproductive success of *C. oppositifolia* and the alpine flora of central Chile in general, future empirical research should manipulate both factors during a number of seasons (Muñoz et al., 2005). This way, we can begin to unravel, not only if pollen and resource limitation are recurrent phenomena in the central Chilean Andes, but also the interplay between them including whether high reproductive output in one season incurs in future reproductive costs.

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