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# Ovule bet-hedging at high elevation in the South American Andes: Evidence from a phylogenetically controlled multispecies study

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## Abstract

1. How animal-pollinated plants support low and stochastic pollination in the high alpine is a key question in plant ecology. The ovule bet-hedging hypothesis proposes compensation for stochastic pollination via ovule oversupply in flowers allowing the benefits of windfall pollination events to be reaped. Under this hypothesis, ovule number is expected to increase from tree line upward on high mountains characterized by steep declines in flower visitation rates and increasingly more variable pollination.

2. Ovule/floret number was investigated for a total of 174 simple-flowered and pseudanthial species in the central Chilean Andes (2,100–3,650 m.a.s.l.). Phylogenetic reconstruction was undertaken using ITS sequences and a constrained ordinal-level backbone reflecting the APG-IV topology. Ovule/floret number was modelled with ordinary least squares regression (OLS) and phylogenetic generalized least squares regression (PGLS) with elevation, floral biomass, life history, pollinator efficiency, pollination generalization, and seasonal flowering period as explanatory variables.

3. The best performing OLS and PGLS models for simple-flowered species consistently included vegetation belt and floral biomass, and with PGLS, pollination efficiency and flowering period. For pseudanthial species, explanatory variables were always floral biomass and its interaction with elevation. Effects of life history and generalized pollination was not found. Ovule/floret number showed high phylogenetic signal, increased with floral biomass and was generally higher in the upper alpine belt in both floral categories. Simple-flowered species with efficient pollination and flowering early, respectively, had larger ovule numbers.

4. *Synthesis*. Ovule number increases with elevation in the central Chilean alpine in two separate floral groups independently of some effects of flowering period and pollinator efficiency. Greater disparity in pollen deposition on stigmas than with inefficient pollination under low visitation rates might explain the association between efficient pollination and higher ovule numbers. Our study provides the first empirical evidence for ovule bet-hedging in the alpine environment. Future studies on the ovule bet-hedging hypothesis should include a measure of flower size.

## 1 | INTRODUCTION

An estimated 87.5% of flowering plants are pollinated by animals (Ollerton, Winfree, & Tarrant, 2011). This percentage translates to over 323,000 species based on the current total of 396,400 angiosperms (Willis, 2016). While it brings many benefits to flowering plants (Mitchell, Irwin, Flanagan, & Karron, 2009), animal pollination is associated with unpredictable variation in the number of pollen grains received by flowers which in turn can lead to disparity in flower-to-flower seed set (Burd, 1995). Such stochastic pollination is driven by the intrinsic behaviour of pollinators (e.g., Jones, 1997), climatic conditions and local weather variation as they affect pollinator survival and activity (Bergman, Molau, & Holmgren, 1996; Corbet, 1990; Torres-Díaz, Cavieres, Muñoz-Ramírez, & Arroyo, 2007; Totland, 1994) and interspecific competition for pollinators leading to asymmetry in visitation intensity among coflowering species (Mitchell et al., 2009). Flower visitation rates are known to vary by at least a factor of 10 across biological communities (Primack & Inouye, 1993). While very high visitation rates can ameliorate the adverse effect of stochastic visitation on seed set, thereby leading to an excess of pollen on a proportion of stigmas, at the other extreme, very low visitation tends to exacerbate it given that a significant number of stigmas are likely to receive little or no pollen at all.

In order to survive in strongly stochastic pollination environments, we expect animal-pollinated plants to possess traits that enable compensation of adverse fitness effects (Arroyo, Muñoz, Henríquez, Till-Bottraud, & Pérez, 2006; Bond, 1994). Ovule oversupply, the production of large numbers of ovules, and considered to represent a bet-hedging strategy, is one such possibility. Bet-hedging is an evolutionary strategy adopted by living organisms to cope with uncertain environments (Philippi & Seger, 1989; Simons, 2011). The ovule bet-hedging hypothesis sees an oversupply of ovules at the level of the individual flower compensating for an absence of seed set in flowers that fail to be pollinated or receive little pollen (Burd, 1995; Burd et al., 2009; Rosenheim, Schreiber, & Williams, 2016; Rosenheim, Williams, & Schreiber, 2014; Schreiber, Rosenheim, Williams, & Harder, 2015). Under stochastic pollination, plants are seen to obtain fitness gains by taking maximum advantage of reproductive opportunities afforded by those flowers that manage to receive ample pollen. The more frequent such windfalls and the greater their magnitude, the greater the ovule number that should be favoured by selection (Burd et al., 2009). In agreement, a phylogenetically controlled analysis of 187 species drawn from the literature found ovule number to be positively correlated with two surrogate measures of degree of flower-to-flower variation in potential mating success (Burd et al., 2009).

Acceptance of ovule bet-hedging as deeply engrained in flowering plants would be greatly strengthened by empirical evidence coming from comparisons of ovule number in plant communities differing in levels of pollination stochasticity. In general, alpine and Arctic habitats are characterized by strongly stochastic pollination as evidenced by variable visitation rates, erratic visitation, wide variation in numbers of pollen grains deposited on stigmas, and large differences in seed set per flower (Ai, Zhou, Xu, Wang, & Li, 2013; Bergman et al., 1996; Eriksen, Molau, & Svensson, 1993; Fulkerson, Whittall, & Carlson, 2012; Hocking, 1968; Kasagi & Kudo, 2003; Kudo & Hirao, 2006; Kudo, Hirao, & Kawai, 2011; Ladinig & Wagner, 2007; Ladinig, Hacker, Neuner, & Wagner, 2013; Lundemo & Totland, 2007; McCall & Primack, 1992; Tiusanen, Hebert, Schmidt, & Roslin, 2016; Torres-Diaz et al., 2007; Totland, 1994, 1997 ; Tur, Sáez, Traveset, & Aizen, 2016; Waites & Ågren, 2004). Progressively shorter growing seasons with increasing elevation and latitude place further restrictions on pollination and seed set by limiting the amount of time available for flowering and seed maturation (Körner, 2003). Alpine areas and the Arctic thus are prime candidates for ovule bet-hedging (Burd et al., 2009). However, although the possibility of ovule oversupply has been mentioned by some authors (Fulkerson et al., 2012; Wagner, Lechleitner, & Hosp, 2016), no study has explicitly tested the ovule bet-hedging hypothesis in these harsh environments for animal pollinators.

Here, we focus on ovule bet-hedging in the high South American Andes, a major alpine area characterized by some of the lowest pollination rates recorded among alpine and terrestrial ecosystems in general (Primack & Inouye, 1993; see also Fulkerson et al., 2012). Several documented elevational trends, taken together, attest to increasingly stochastic pollination from tree line upward in the southern South American Andes, among them progressively impoverished pollinator assemblages (Arroyo, Primack, & Armesto, 1982; Medan et al., 2002; Squeo, Cepeda, Olivares, & Arroyo, 2006), deteriorating pollination network structure (Ramos-Jiliberto et al., 2010), severe declines in community-wide visitation rates on three alpine gradients spread over 32° of latitude (Arroyo & Squeo, 1990; Arroyo, Armesto, & Primack, 1985), and reductions in the proportion of species visited or flowers forming fruits (Arroyo, Pacheco, & Dudley, 2017; Medan et al., 2002). Moreover, where looked at, pollen receipt on stigmas has been found to vary over two orders of magnitude with large numbers of stigmas receiving no pollen at all (Tur et al., 2016). Additionally, within species, lower visitation rates and/or smaller stigmatic pollen loads have been found in higher elevation populations of strongly and facultatively outcrossing species with large elevational ranges (Arroyo et al., 2017; Medan, 2003; Seguí, Lázaro, Traveset, Salgado-Luarte, & Gianoli, 2018).

Contrasting with the above scenario, counterintuitively, alpine communities in the Andes contain many showy-flowered species that are totally or strongly dependent on animal pollination (Arroyo & Squeo, 1990; Arroyo, Humaña, Domínguez, & Jespersen, 2012; Ladd & Arroyo, 2009; Medan, 2003; Medan et al., 2002; Muñoz & Arroyo, 2006; Muñoz & Cavieres, 2008; Pérez, Arroyo, & Armesto, 2009; Torres-Díaz et al., 2011; Tur et al., 2016) with no decrease in self-incompatibility and sexual dimorphism over elevation detected on one well-studied alpine gradient (Arroyo & Squeo, 1990). This constellation of characteristics suggests reduced and less predictable pollination in the upper alpine does not severely limit seed set. In several strongly outcrossing high Andean species, less reliable visitation on higher sites is known to be ameliorated by intrinsically long-lived flowers of capitula in Asteraceae or more prolonged flower longevity produced by the plastic extension of the flower life span under cooler temperatures and higher soil moisture content allowing more time for the accumulation of scarce pollinator visits (Arroyo et al., 2017; Arroyo, Dudley, Jespersen, Pacheco, & Cavieres, 2013; Dudley, Arroyo, & Fernández-Murillo, 2018; Medan, 2003; Pacheco, Dudley, Cabezas, Cavieres, & Arroyo, 2016; Torres-Díaz et al., 2011). Long-lived flowers have also been implicated in compensating low and stochastic visitation in several other alpine areas (Ai et al., 2013; Bingham & Orthner, 1998; Duan, & Liu, 2007; Pickering, 1997; Steinacher & Wagner, 2010; Utelli & Roy, 2000). However, in the Andes, flower longevity compensation is rarely fail-safe as evidenced by significant pollen limitation in most strongly outcrossing species studied to date with supplemental pollination and higher levels of pollen limitation in upper elevation populations of individual species (Arroyo et al., 2017; Ladd & Arroyo, 2009; Muñoz & Arroyo, 2006; Muñoz & Cavieres, 2008; Torres-Díaz et al., 2011). Under these circumstances, ovule oversupply as an additional means for counteracting the negative seed set effects of stochastic pollination becomes a plausible hypothesis.

The outstanding variation in ovule number in flowering plants covering six orders of magnitude (Burd, 1995) is likely to be partially due to differences in pollination mechanisms and breeding systems. If so, with significant pollinator turnover along the alpine gradient as occurs in the Andes (Arroyo & Squeo, 1990; Arroyo et al., 1982), pollination can be expected to contribute to variation in ovule number. It is well known that different pollination vectors differ widely in the amount of pollen they transport (e.g., Castellanos, Wilson, & Thomson, 2003; Herrera, 1987; Mayfield, Waser, & Price, 2001; Medan, Zarlavsky, & Bartoloni, 2013; Saunders, Peisley, Rader, & Luck, 2016; Wiklund, Eriksson, & Lundberg, 1979). As a case in point, Mizunaga and Kudo (2017) showed that inefficient dipterans must make three times as many visits as efficient bees to achieve comparable fruiting success, implying substantial differences in the amount of pollen deposited on stigmas. It is interesting to ask, therefore, whether ovule number differs among species with efficient and inefficient pollinators. Level of pollination generalization could also be relevant. Because many different pollinators are involved, variation in the number of pollen grains received on stigmas under generalized pollination is likely to be less pronounced than

with specialized pollination, leading to the prediction of lower ovule numbers. Moreover, generalized pollination allows plant species to reap the benefits of pollinators being active over a greater proportion of environmental variability than specialized pollination (Waser, Chittka, Price, Williams, & Ollerton, 1996) and is also known to be associated with lower levels of pollen limitation (Pérez et al., 2009; Waser, et al., 1996). Therefore, overall, more stigmas are likely to receive at least some pollen than with specialized pollination. For breeding system, intuitively, ovule oversupply is most expected in strongly pollinator-dependent self-incompatible species. Stigmas of flowers in highly autogamous species will receive similar amounts of pollen, whereas at the other extreme, in highly self-incompatible species, much variation in stigmatic pollen loads is expected. Such variation will have the greatest impact on seed set when pollination rates are very low and consequently should favour windfall pollination events under such conditions.

In addition to the last-mentioned factors, some studies have found ovule number to increase with flower size (Davis, 1981; López, Rodriguez-Riano, Ortega-Olivencia, Devesa, & Ruiz, 1999; Wetzstein, Yi, Porter, & Ravid, 2013; see also Chalcoff & Aizen, 2016). A rigorous test of the ovule bet-hedging hypothesis over elevation requires taking flower size into account in order to discard the possibility that larger ovules numbers are simply a correlate of larger flowers selected to increase pollinator visitation or of elevational turnover in pollination mechanisms which is known to be associated with changes in flower size (e.g., Maad, Armbruster, & Fenster, 2013). Finally, pollination rates in the alpine (the central Chilean Andes included) tend to be lower and more variable early in the flowering season before pollinator populations have built up and due to spatiotemporal variation in snow melt (Arroyo et al., 1985; Hirao, Kameyama, Ohara, Isagi, & Kudo, 2006; Kudo, 1993). As over elevation, larger numbers of ovules are expected in early flowering species.

To assess the effect of elevation and other variables on ovule number in the southern Andes, we investigated ovule number in 174 species found over a long alpine gradient in central Chile comprising two vegetation belts (33°S) using a phylogenetically controlled multispecies approach. Species included both typical simple-flowered species and pseudanthial species as in Asteraceae. The latter on the grounds that pseudanthia perform an equivalent function to simple flowers in terms of pollinator attraction (Weberling, 1998). In view of likely phylogenetic effects, we built comprehensive phylogenetic trees for species in both floral categories. Using ordinary least squares regression (OLS) and phylogenetic generalized least regression (PGLS) ,we modelled ovule number considering elevation, two pollination variables, seasonal flowering time, floral biomass, and life history as a proxy for breeding system as explanatory variables, and compared flower/pseudanthium size at different elevations. Our main hypothesis was that plant species in the upper alpine in the central Chilean Andes are expected to show higher ovule numbers per flower compared to their lower subalpine counterparts. Ideally, to test the ovule bet-hedging hypothesis, data on flower-to-flower variation in pollen receipt on stigmas and seed set is required (cf.,

Burd, 1995). However, as seen in the mere 187 records that were available across all habitat types at the time of Burd et al. (2009), this kind of information is extremely difficult to obtain for large numbers of species, let alone for a very large number of species on a single site, as considered here. Therefore, as the next best option, we resorted to looking for broad ecological pattern in ovule number.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

Work was carried out in the subalpine (sometime referred to as the lower alpine) and high alpine vegetation belts in the Farellones, Valle-Nevado, and La Parva area immediately to the east of Santiago (33°S). Both of these two vegetation belts occur above tree line. In this latitude of the Chilean Andes, the alpine gradient is unusually long (2,100–3,750 m.a.s.l.) on account of the combined effect of a naturally depressed mediterranean-climate tree line due to aridity (Piper et al., 2016) and a high summer 0°C isotherm lying at over 4,000 m.a.s.l. (Carrasco, Casassa, & Quintana, 2005). Choice of a long alpine gradient facilitated avoidance of slope/aspect and local topography effects that can confound regional elevational trends when short alpine gradients are considered (Arroyo et al., 2013). According to the nearest high elevation weather stations, mean air temperature for the warmest period of the year (October–March) is 12.4°C at 2,475 m.a.s.l. (El Yeso Embalse) and 9.4°C at 2,780 m.a.s.l. (Laguna Negra) ( https://explorador.cr2.cl/). Based on the monthly temperature lapse rates for the Valle Nevado area (Cavieres & Arroyo, 1999), mean air temperature for the warmest months is 3.2°C at 3,750 m.a.s.l. Cloudy days increase in frequency with elevation (Viale & Garreaud, 2014) as does intermittent afternoon cloudiness and windiness leading to the more variable temperatures at higher elevations.

The subalpine belt as defined here (hereafter LOW) occurs from the ecotone with the open *Kageneckia angustifolia* tree line at 2,100–2,200 m.a.s.l. to 2,650–2,800 m.a.s.l. (exact elevational limit depends on exposition). The succeeding high alpine belt (hereafter HIGH) continues to 3,750 m.a.s.l. LOW is comprised of low rounded shrubs, perennial herbs, and annual herbs. HIGH is dominated by a wide band of cushion plants and perennial herbs which eventually give way to scattered perennial herbs and subshrubs in its upper reaches. Occasional annuals can still be found to as high as 3,500 m.a.s.l. The two alpine belts are strongly floristically differentiated with higher species richness in the lower belt (Cavieres, Peñaloza, & Arroyo, 2000).

Flowering begins from around mid-September on the lower end of the study area to end in late April in the upper reaches with the mean length of the flowering period for individual species increasing over the alpine gradient (Arroyo, Armesto, & Villagran, 1981). Pollination in the study area is predominantly by bees, butterflies, and flies, with a few species visited by hummingbirds, ants, and coleopterans (Arroyo et al., 1982). The estimated

17% anemophilous species comprise mostly Poaceae, Juncaceae, and Cyperaceae. Bee pollination declines while butterfly and fly pollination increase in importance with increasing elevation (Arroyo et al., 1982). On this particular alpine gradient, flowers in the upper alpine receive only around half as many visits per unit time as those in the lower subalpine (Arroyo et al., 1985, 2017). Temperature fluctuation over short time-scales relevant to pollinator visitation and visitation rates become more variable with increasing elevation (Arroyo, et al., 1985; Supporting Information Material S1).

### 2.2 | Field sampling

We collected material for ovule counts and floral biomass determination for 130 simple-flowered species (hereafter SF species) and 44 pseudanthial species (hereafter PS species). A full listing of the species studied can be found in the Dryad Digital Repository (Arroyo et al., 2018) and in Supporting Information Material S3. One hundred and sixteen species pertain to LOW and 58 to HIGH. *Cerastium arvense* L. was included given some doubt over the exotic status of high mountain plants identified as this species in the southern Andes. Sampling was carried out across the full austral summer flowering season by two to three persons who combed the entire alpine gradient. Intensive sampling assured adequate representation of the floristic composition of each vegetation belt, elevational differences in species richness, and the range of flowering times at different elevations. Most species on our study sites are found exclusively or are preferentially distributed in one of the two alpine belts and were sampled in that vegetation belt. So as to comply with the limitations of the Comparative Method, only one population was sampled for the few species distributed across the entire alpine gradient; in most cases that population came from the vegetation belt where the species' abundance was higher. Species suspected to be exclusively wind-pollinated were not included, given that elevational and seasonal differences in ovule number are not expected. Moreover, ovule number in grasses, which predominate among wind-pollinated species on our study site, is phylogenetically fixed at one (Judd, Campbell, Kellog, Stevens, & Donoghue, 2008). Two species of the genus *Acaena* which is known to be visited by insects but which appears also to be facultatively wind-pollinated (Arroyo et al., 1982) were admitted.

### 2.3 | Ovule number and floral biomass

We collected two neighbouring fresh flowers (or pseudanthia) on a targeted 20–25 individuals per species. One flower/pseudanthium was preserved in 70% ethanol and destined for ovule counts under a dissecting microscope. The second was stored in a paper sack for floral biomass determination. For the latter, material was dried at 70°C for 48 hr in a laboratory oven and weighed on a 0.0001 g precision balance (Denver Instrument Company). In some asteraceous species, we counted ovule numbers directly in the field and obtained dry weights for the same dismembered heads. A few SF

species bore a single flower. For these, we collected the second flower from a neighbouring plant. Some very high-elevation species form tightly interwoven mats making it difficult to identify individual plants. In these cases, the two flowers collected will not necessarily be from identical individuals. We had difficulty counting the thousands of ovules in *Caiophora coronata* (Loasaceae) due to their tendency to clump together. For this species, we used a conservative estimate of ovule number based on available seed count data. In one asteraceous species (*Haplopappus schumannii*), where all capitula were heavy insect-infested, we used an estimate of total floret number per capitulum obtained from crossing experiments (M. T. K. Arroyo, unpublished data). One species of *Nassauvia* has fairly tight compound heads which we originally considered the pollination unit. However, given that a compound head is not morphologically equivalent to a single head, in the final analysis, we opted to use the single head to be consistent with other Asteraceae and species in this genus. Overall, we obtained ovule number and floral biomass on 20–25 individuals for 84.5% of the sampled species. Species with lower final sample sizes either suffered losses due to ovule infection or lacked sufficient individuals with two recently opened flowers or pseudanthia at the time of sampling.

## 2.4 | Flowering phenology

To test for an effect of flowering time on ovule number, we divided the flowering season into early-, middle-, and late-flowering based on phenological curves for the same area given in Arroyo et al. (1981). The middle part of the flowering season was considered to run from around the beginning of December to the end of January at 2,320 m.a.s.l., mid-December to the end of February at 2,700 m.a.s.l. (10°C), and the beginning of January to the end of March at 3,550 m.a.s.l. Species were assigned to one of the three flowering periods based on their peak flowering dates. Peak flowering dates for 70 of the 174 species (32 high, 38 low) could be obtained directly from Arroyo et al. (1981). For the remaining species, we estimated peak flowering dates by calculating the length of the flowering period for the elevation at which each species was studied. The length of a species' flowering period was interpolated for a given elevation using an average of 4.2 weeks at 2,320 m.a.s.l. and 8.4 weeks at 3,550 m.a.s.l. (Arroyo et al., 1981). We then pinpointed the midpoint flowering date, assuming that flowering had been going on for at least 10 days at the time we collected floral material. To assess the accuracy of our estimations, we compared the available published peak flowering dates (*n* = 70) with their estimated peak flowering dates, obtaining identical results in 88.6% of the cases. In all eight noncoincident species, the estimated flowering peak fell into an earlier seasonal category. Although many species continue to flower in the late part of the flowering season, only two species, both from LOW, showed peak flowering over that period. In view of the fact that visitation rates are still relatively high at this time of the year (Arroyo et al., 1985), these species were placed with the mid-season species.

## 2.5 | Pollination

For degree of pollination generalization, following Lázaro, Hegland, and Totland (2008) (see also Sahli & Conner, 2006), we used the Simpson Diversity Index: $1/D = 1/\sum_{i=1}^{S} p_i^2$ where $p_i$ is the proportional visitation for pollinator group *i* and *S* the number of pollinator groups (e.g., dipterans, lepidopterans, etc.) visiting a plant species. Highly specialized pollination takes the value of one while increasingly larger values indicate increasing generalization. For two species of the specialized bee-pollinated genus *Calceolaria*, we assumed that reported bees were the only pollinators. For pollinator efficiency, we determined the pollinator group responsible for the highest proportion of visits to a species and classified it as efficient or inefficient. Bees and hummingbirds were considered efficient pollinators while lepidopterans, dipterans, coleopterans, and ants were considered inefficient pollinators. All information for pollination came from the literature (mainly from earlier work by one of us in the same area—Arroyo et al., 1982) where either proportional visitation was reported or could be calculated. Overall, we obtained useful pollinator information for 109 species (LOW = 69; HIGH = 40). Seven additional species were reported as not receiving visits.

## 2.6 | Life history

Presently, there are insufficient published breeding system records for the central Chilean Andes to investigate the effect of breeding system on ovule number. We therefore opted for an indirect approach that relied on the trend for autogamy and self-compatibility (including in alpine floras) to be more strongly represented in annual than perennial species (Arroyo & Squeo, 1990; Arroyo & Uslar, 1993; Razanajatovo et al., 2016; Wiens, 1984). Accordingly, if breeding system is important, overall, we expect to find a tendency for higher ovule numbers in perennial species. Species were categorized as annual (annual herbs) and perennial species (perennial herbs, subshrubs, suffrutices, and shrubs). A few of the annual herbs can also be biennials.

## 2.7 | Phylogenetic reconstruction

We constructed separate Bayesian inference molecular phylogenies for SF and PS species using the nuclear ITS locus. ITS is a fast-evolving locus that is useful for inferring phylogenetic relationships at lower taxonomic levels (Mort et al., 2007). Good resolution of the tips of the phylogenies was desirable as several genera in our samples are represented by more than one species (as many as nine species in *Adesmia* and eight in *Senecio*). For 52 species (29.9%), we accessed ITS sequences from GenBank. For *Blumenbachia dissecta*, we used a sequence for *Blumenbachia sylvestris*. For *Pyrrhocactus curvispinus*, often considered under *Eriosyce*, we used *Eriosyce subgibbosa*. In both cases where such surrogate sequences were used, the genus is represented by a single species in our samples. For the remaining 122 species (70.1%), DNA was extracted in our laboratory from samples collected in the field on our sites. Details of laboratory

protocols, phylogenetic reconstruction, and GenBank accession numbers can be found in Supporting Information Materials S2 and S3.

The ITS topologies were generally coherent with respect to placement of species in their respective genera and families. However, the deeper branches of the SF tree did not always reflect their positions as per the APG-IV topology (APG IV, 2016), considered to represent the best available knowledge on the phylogenetic relationships of deeper angiosperm lineages. We therefore constrained the ordinal-level branches of the SF tree to reflect the APG-IV topology. Separate Bayesian inference phylogenetic trees were built for SF and PS species using MrBayes 3.2 (Ronquist et al., 2012) and the functions "constraints" and "topologypr." A General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites (GTR+I model) was used. We conducted two independent runs of 10,000,000 generations and sampled every 1,000 generation with a burn-in fraction of 0.25. Consensus trees computed from sampled trees were made ultrametric using the "chronos" function in the APE package version 3.1 3 (https://cran.r-project.org/src/contrib/PACKAGES.html#ape) (Paradis, Claude, & Strimmer, 2004). Polytomies detected in the consensus trees were resolved randomly assigning a branch length equal to 0.0001.

## 2.8 | Data analysis

Ovule/floret number and floral biomass were the means for the individuals sampled and were log (ln) transformed for statistical purposes. The effect of vegetation belt and other variables on ovule/floret number was examined using PGLS. PGLS was carried out using a correlation structure that accounts for phylogenetic dependencies between species based on the Pagel lambda index ($\lambda$). $\lambda = 0$ indicates phylogenetic independence, $\lambda = 1$ indicates that species covary as predicted by a Brownian motion model of evolution, and $\lambda > 1$ indicates more covariance than expected under a Brownian model. PGLS was performed using the function corPagel in the APE r package (Paradis et al., 2004) in connection with the function gls of the nlme r package (Pinheiro, Bates, DebRoy, & Sarkar; R Core Team, 2018). Ovule/floret number was also modelled assuming phylogenetic independence using the same r functions described above but setting $\lambda$ to 0. This last analysis is equivalent to OLS when trees are ultrametric (Orme, 2018).

The best-fit models were identified using a stepwise model selection procedure based on Akaike's information criterion (AIC) using the function stepAIC in the Mass Package implemented in r (Venables & Ripley, 2002). This procedure starts with a full model which includes all possible candidate explanatory variables and sequentially removes or adds one variable at each step to find the model with the lowest AIC value. Our first analyses considered all variables that could be analysed for the total number of species (174) sampled. For SF species, the initial model in the stepwise procedure included vegetation belt, floral biomass, life history, and flowering period along with all first-order interactions between vegetation belt and the other variables considered. For PS species, the initial model included the same variables, except that flowering period was excluded as there were no early flowering species on HIGH and all but two species fell into the mid-season flowering category on LOW. Second PGLS and GLS analyses were performed on those species for which data on pollinator efficiency and pollinator generalization were available (SF species, $n = 109$; PS species, $n = 29$). For SF species, initial models in the stepwise procedure included all previously considered explanatory variables, the two pollination variables and their respective interactions with vegetation belt. For PS species, variables were the same with the exception that flowering period again was not considered. The models selected by the stepwise procedure for PS species included nonsignificant terms. We removed those variables to build the final OLS and PGLS models (resulting in lower AIC values). Additionally, we analysed floral biomass for SF and PS species with OLS and PGLS to determine whether flower and pseudanthium size increases with elevation.

## 3 | RESULTS

The 174 species sampled belong to 36 plant families and 93 genera and thus constitute a taxonomically diverse set of species. The five most abundant plant families in our samples are Asteraceae (43 spp.), Fabaceae (15 spp.), Amaryllidaceae (9 spp.), Montiaceae (9 spp.), and Brassicaceae (7 spp.). Other well-represented families are Calceolariaceae, Polemoniaceae, Oxalidaceae, Caryophyllaceae, Apiaceae, and Boraginaceae. SF species belong to 76 genera and 34 families; PS species to 17 genera and two families (Asteraceae and Calyceraceae). Calyceraceae is an endemic South American family that is sister to Asteraceae (Pozner, Zanotti, & Johnson, 2012). As in Asteraceae, the individual florets of the pseudanthium in this family possess a single ovule. The overall number of species sampled is considerably larger than the 103 vascular plant species (Poaceae and exotic species included) reported in a gradient study in the Valle Nevado area (Cavieres et al., 2000) and thus is highly representative of the alpine flora for what is a large study area. The large numerical difference is partially a reflection of the inclusion in our study of 20 species from wet habitats, not considered in the above-mentioned study.

Generalized and specialized pollination are found in both vegetation belts. However there was no significant difference in Simpson´s Diversity Index (Mann–Whitney $U$-test: $p = 0.841$) among vegetation belts. Efficient pollination was far more frequent in the lower part of the alpine (LOW = 56.5%; HIGH = 27.5%; Test of Proportions: $p < 0.005$) where bee of pollination is more common. Not surprisingly, given the open nature of pseudanthia, pollination in PS species was significantly more generalized than in SF species (Mann–Whitney $U$-test: $p < 0.001$). Although there was a clear tendency for more species with efficient pollinators among SF species, the difference was not significant (SF = 48.8%; PS = 37.9%; Test of Proportions: NS). Not surprisingly, annual species were more abundant on LOW (25.0%) than on HIGH (8.6%) (Test of Proportions:
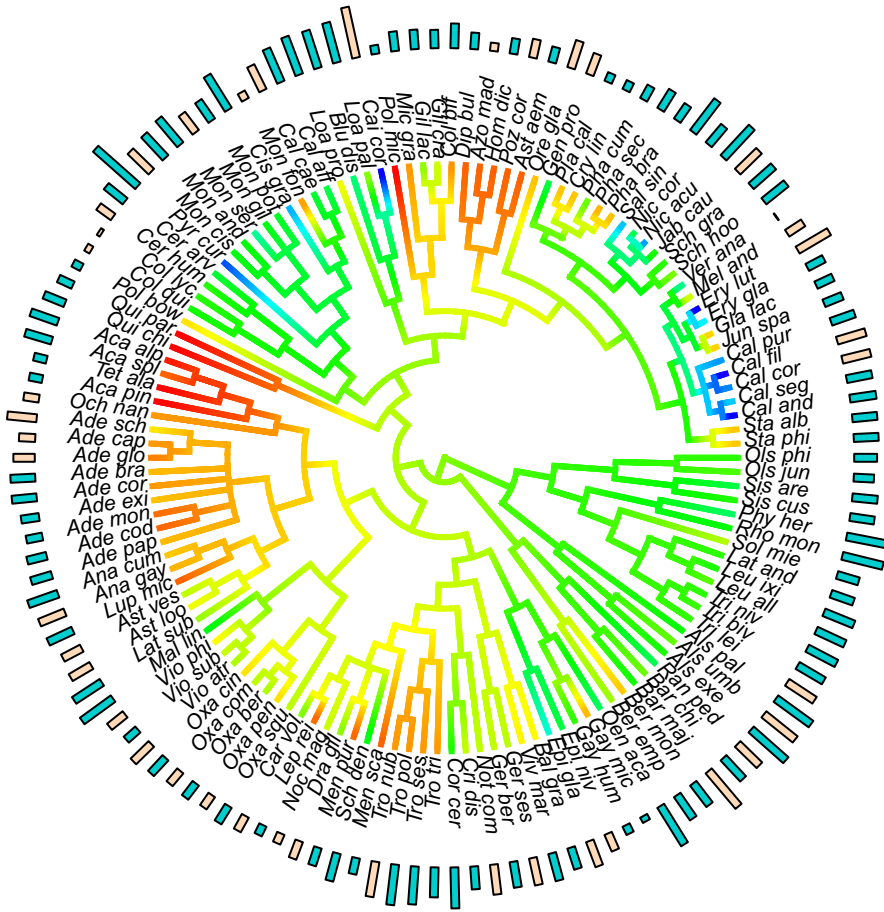
**FIGURE 1** Phylogenetic tree for simple-flowered species (SF) showing ln ovule number per flower (branch colour) and ln floral biomass (bar length) external to species names. Branches: cold colours indicate high ovule numbers; warm colours indicate low ovule numbers. Bars: light brown indicate subalpine (LOW); blue bars indicate upper alpine (HIGH). Full names for species can be found in the Dryad Data Repository (Arroyo et al., 2018) and in Supporting Information Material S3

$p < 0.05$). Flowering in 70.1% of the species occurred in the middle of the flowering season. In relative terms, proportionally fewer species on HIGH (15.5%) than on LOW (37.0%) showed peak flowering in the early part of the flowering season for the vegetation belt (Test of Proportions: $p < 0.005$).

## 3.1 | Simple-flowered species

Mean ovule number varied from 1 (several species) to 2,140.3 (*Erythranthe lutea*) and was variable in 74.6% cases. Floral biomass ranged from 0.15 to 475.1 mg. Invariant ovule numbers were concentrated in the Apiaceae, Lamiaceae, and Boraginaceae. The proportions of species with an invariant ovule number on LOW (25.6%) and HIGH (25.0%) were not significantly different (Test of Proportions: $Z = 0.067$, $p = 0.944$). Ovule number per flower in invariant species was also not significantly different between HIGH and LOW (Mann–Whitney $U$ test: $Z = 1.900$, $p = 0.057$). Figure 1 shows the constrained phylogenetic tree for SF species along with the distribution of ovule number and floral biomass. It can be seen that ovule number and floral biomass are not uniformly distributed over the tree. PGLS revealed fairly strong phylogenetic signal for floral biomass ( = 0.86). There was no significant difference in floral biomass between the two vegetation belts (OLS: $t_{1,128} = -0.88$, $p = 0.38$; PGLS: $t_{1,128} = -0.22$, $p = 0.88$).

For the first analyses on the full set of species, for both OLS and PGLS, the best performing models on ovule number included floral biomass and vegetation belt as explanatory variables (Table 1). Life history, flowering period, and all interactions had no effects. The fit of the best PGLS model improved with respect to OLS (see lower AIC value for PGLS, Table 1). Reflecting the unequal distribution of ovule numbers on the phylogenetic tree (Figure 1), ovule number showed moderately high phylogenetic signal ($\lambda = 0.68$; Table 1). Results show that elevation and floral biomass both had positive effects on ovule number (Table 1). As can be seen in Figure 2, for any given floral biomass, HIGH flowers possess more ovules than LOW flowers. Overall, these results for SF species are in strong agreement with the ovule oversupply hypothesis.

For second analyses on SF species where we were able to analyse pollination efficiency and level of pollination generalization in addition to the earlier variables, the best OLS and PGLS models again included elevation and floral biomass, but not life history, with identical trends as described above. However, PGLS now revealed significant effects of flowering period, pollinator efficiency, and the interaction between elevation and pollinator efficiency. Level of pollination generalization had no significant effect either with OLS or PGLS. As with the analyses on the full set of species, ovule number showed high phylogenetic signal ($\lambda = 0.91$) (Table 1) and the fit of the PGLS model improved with respect to OLS (see Aike values in

**TABLE 1** Statistics for the best OLS and PGLS models for ovule number in simple-flowered (SF) and pseudanthial (PS) species obtained using the stepwise selection AIC procedure. Initial models included all explanatory variables for which data were available and all first-order interactions with vegetation belt. Explanatory variables were: VB, vegetation belt (LOW, HIGH); FB, floral biomass (ln); LH, life history (annual, perennial); FP, flowering period (early, mid); PE, pollinator efficiency (low, high); PG, pollinator generalization. For categorical variables, estimates and t-values refer to the first-mentioned state in parenthesis. All data can be found in the Dryad Digital Repository (Arroyo et al., 2018)

| Simple-flowered species (SF): Complete dataset (n = 130 species) | |
|---|---|
| OLS ($\lambda$ = 0) | PGLS ($\lambda$ = 0.68) |
| *Best model:* FB + VB | *Best model:* FB + VB |
| AIC = 488.9 | AIC = 442.8 |
| FB: 0.56, t = 6.15, p < 0.001 | FB: 0.58, t = 6.89, p < 0.001 |
| VB: 0.59, t = 1.98, p = 0.049 | VB: 0.48, t = 2.16, p = 0.03 |
| Simple-flowered species (SF): Partial dataset (n = 80 species) | |
| OLS ($\lambda$ = 0) | PGLS ($\lambda$ = 0.91) |
| *Best model:* FB + VB | *Best model:* FB + VB + PE + FP + VB × PE |
| AIC = 295.6 | AIC = 260.4 |
| FB: 0.78, t = 6.14, p < 0.001 | FB: 0.66, t = 5.06, p < 0.001 |
| VB: 0.77, t = 2.18, p = 0.03 | VB: 1.79, t = 4.44, p < 0.001 |
| | PE: 1.90, t = 3.64, p = 0.005 |
| | FP: −0.73, t = −2.65, p = 0.010 |
| | VB × PE: 1.12, t = 2.19, p = 0.03 |
| Pseudanthial species (PS): Complete dataset (n = 44 species) | |
| OLS ($\lambda$ = 0) | PGLS ($\lambda$ = 1) |
| *Best model:* FB + VB + FB × VB | *Best model:* FB+VB+FB × VB |
| AIC = 112.7 | AIC = 86.6 |
| FB: 0.56; t = 3.25, p = 0.002 | FB: 0.44; t = 4.69, p < 0.001 |
| VB: −1.44, t = −1.74, p = 0.09 | VB: −0.41, t = −0.96, p = 0.34 |
| FB × VB: 0.47, t = 2.23, p = 0.03 | FB × VB: 0.24, t = 2.07, p = 0.04 |
| Pseudanthial species (PS): Partial dataset (n = 29 species) | |
| OLS ($\lambda$ = 0) | PGLS ($\lambda$ = 1) |
| *Best model:* FB + VB | *Best model:* FB + VB |
| AIC = 73.0 | AIC = 63.5 |
| FB: 0.37, t = 3.08, p = 0.005 | FB: 0.33, t = 3.45, p = 0.002 |
| VB: 0.58, t = 1.91, p = 0.06 | VB: 0.58, t = 2.75, p = 0.01 |

Table 1). The PGLS results indicate that ovule number is not only larger in the upper alpine but also in early-flowering species as we originally predicted. Additionally, efficient pollination was associated with higher ovule numbers. Moreover, as shown by the significant interaction between elevation and pollinator efficiency, this effect became more exaggerated on HIGH. The consistency of the results for elevation and seasonal flowering period in SF species is impressive and provides strong support for the ovule bet-hedging hypothesis.

## 3.2 | Pseudanthial species

All PS species showed variation in floret number except one. Mean floret number varied from 4.9 (*Nassauvia axillaris*) to 169.1 (*Hypochaeris thrincioides*) and florets in all species had a single ovule. Floral biomass ranged from 1.4 to 543.9 mg. Figure 3 shows the

constrained phylogenetic tree for PS species along with the distribution of floret number and floral biomass. As with SF species, it can be seen that ovule number and floral biomass are not uniformly distributed over the tree. In accordance, PGLS, as for SF species, revealed strong phylogenetic signal for floral biomass ( = 0.93). OLS and PGLS revealed no significant difference in PS floral biomass between the two vegetation belts (OLS: $t_{1,42}$ = −0.42, p = 0.68; PGLS: $t_{1,42}$ = 1.67, p = 0.10).

In the first analyses on the full set of PS species, the best OLS and PGLS models for ovule number included floral biomass and the interaction between floral biomass and vegetation belt (Table 1). Life history was not relevant. Again, the fit of the PGLS model improved with respect to the OLS model (see lower AIC value, Table 1). Ovule number showed high phylogenetic signal ($\lambda$ = 1) (Table 1, see also Figure 3). Results show that floret number increases with elevation over most of the range of pseudanthium
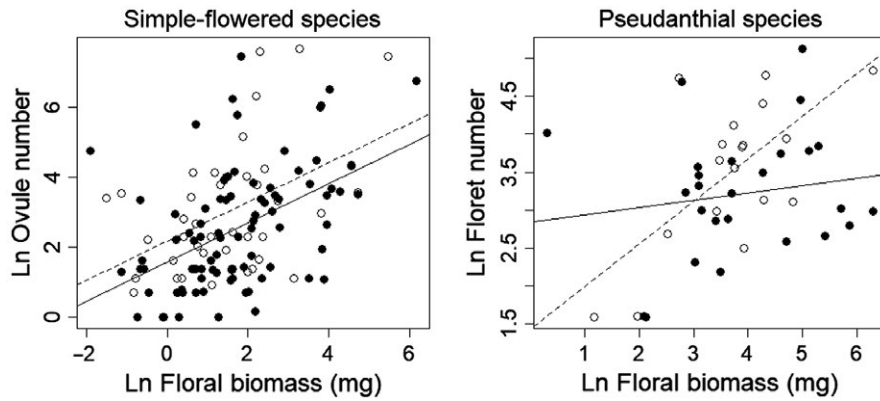
**FIGURE 2** Relationship between ovule number and floral biomass for simple-flowered (SF) and pseudanthial species (PS) in the subalpine (LOW) and high alpine (HIGH) in the central Chilean Andes. HIGH = open circles: LOW = closed circles: dashed lines = HIGH; solid lines = LOW. Lines were fitted according to the best OLS models
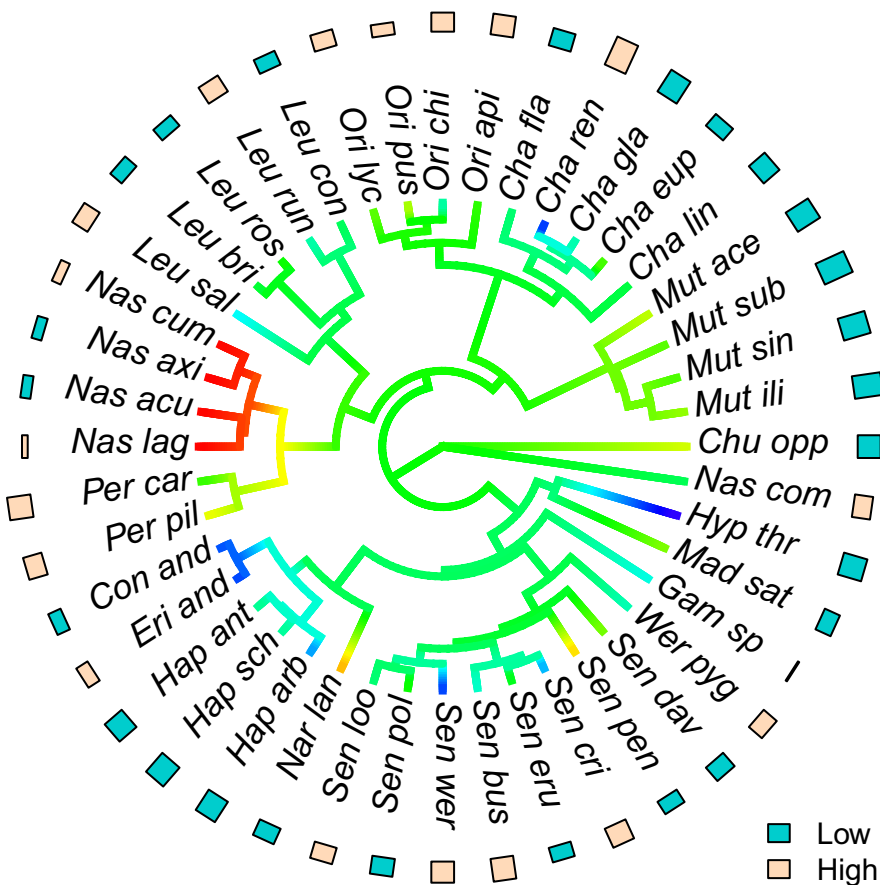


**FIGURE 3** Phylogenetic tree for pseudanthial species (PS) showing ln floret number per pseudanthium (branch colour) and ln floral biomass (bar length) external to species names. Branches: cold colours indicate high ovule numbers; warm colours indicate low ovules number. Bars: light brown indicate subalpine (LOW); blue bars indicate upper alpine (HIGH). Full names for species can be found in the Dryad Data Repository (Arroyo et al., 2018) and in Supporting Information Material S3

sizes in study area (Figure 2). In agreement with the significant interaction with vegetation belt, very large pseudanthia on HIGH showed an exaggerated number of ovules for an equivalent floral biomass on LOW.

For the second PS analyses where the pollination variables were included, the best OLS and PGLS models continued to include floral biomass and its interaction with vegetation belt (Table 1), there being no effect again of life history nor of pollination generalization and efficiency. The PGLS model showed the best fit (see AIC value in Table 1) which is consistent with very high phylogenetic signal found in this analysis ($\lambda = 1$) (Table 1). Overall, results for PS species, with some nuance, provide good support for the ovule bet-hedging hypothesis.

## 4 | DISCUSSION

Bet-hedging is an evolutionary strategy that enables organisms to cope with environmental uncertainty (Philippi & Seger, 1989; Simons, 2011). In plants, seed bank formation (Aslan, Zavaleta, Tershy, & Croll, 2013; Clauss & Venable, 2000; Evans, Ferriere, Kane, & Venables, 2007) and ovule oversupply (Burd, 1995; Burd et al., 2009) are the best documented cases of bet-hedging, but in relative terms, ovule bet-hedging has received far less attention. Chalcoff and Aizen (2016) examined ovule number for populations of a tree species across a rainfall gradient; however, no study has investigated ovule oversupply across a pollination stochasticity gradient at a broad community level as we set out to do here. For simple-flowered

species, we obtained highly consistent evidence for higher numbers of ovules per flower in the upper alpine in the central Chilean Andes. We also found higher floret numbers per pseudanthium over most of the range of pseudanthium sizes represented. Both ovule and floret number showed high phylogenetic signal in both floral categories, as has been found for some other reproductive traits in flowering plants (e.g., Alcantara & Lohmann, 2011; Vandelook, Verdu, & Honnay, 2012; Yang et al., 2014). Although floral biomass did not increase with elevation, larger flowers and pseudanthia tended to have more ovules and florets respectively.

In our PGLS analysis for simple-flowered species where two pollination variables could be incorporated, flowering period and pollinator efficiency appeared in the best model along with floral biomass and elevation. Higher numbers of ovules characterized early-flowering species subject to the inevitable vicissitudes of interannual variation in the time of snow melt and characterized by overall lower pollination rates (Arroyo et al., 1985). The remarkable coincidence between this result and the elevational trend in ovule number adds an exciting new dimension to ovule oversupply. However, caution is called for because this result only emerged in one of the two PGLS simple-flowered analyses. Turning to pollination efficiency, in simple-flowered species, efficient pollination was found to be coupled to higher ovule numbers. Moreover, this tendency was more strongly manifest in the upper alpine. These results are intriguing given that efficient pollination is known to be associated with lower levels of pollen limitation than inefficient pollination (Gómez, Abdelaziz, Lorite, Munoz-Pajares, & Perfectti, 2010; Koski, Ison, Padilla, Pham, & Galloway, 2018). They could find an explanation in the relative amounts of pollen deposited on stigmas by efficient versus inefficient pollinators. In an alpine area like the central Chilean Andes where pollination rates are especially low and variable, greater disparity in stigmatic pollen deposition is likely in species pollinated by efficient pollinators because relatively more pollen will be deposited on the stigmas of flowers that are visited in comparison with those that fail to be visited. Moreover, the effect is expected to increase with elevation, which is precisely what we found. No effect of pollinator efficiency was detected in pseudanthial species, perhaps because pollination mechanisms are much more uniform in this floral category

Two surprising results coming out of our study were the lack of a perceptible effect of degree of pollination generalization and life history on ovule number. We argued that generalized pollination should lead to lower disparity in the number of pollen grains deposited on stigmas than specialized pollination on account of a larger range of pollinators and pollen size loads and associated lower levels of pollen limitation (Knight et al., 2005; Lázaro, Lundgren, & Totland, 2015; Márten-Rodriguez & Fenster, 2010; Wolowski, Ashman, & Freitas, 2014). In other words, higher ovule numbers would be expected with specialized pollination. Lack of an effect of level of pollination generalization on ovule number could have been influenced by the fact that many species in the central Chilean alpine, especially pseudanthial species, have strongly generalized pollination. Moreover, species in both floral classes broadly classed as specialized on the basis

of their main pollinator groups can be pollinated by more than one species for the pollinator group in question and thus are generalized at another level. A finer analysis would be worthwhile.

With respect to life history, annuals are predominantly self-pollinating (Snell & Aarssen, 2005; Wiens, 1984) while perennials include many outcrossing species. Therefore, we expected higher ovules number in perennials. Interestingly, Burd et al. (2009) likewise failed to find an effect of life history on ovule number. Furthermore, ovule number deduced from Wien´s (1984) dataset on seed/ovule ratios (S/O) and brood size (number of seed per fruit) for close to 200 species shows little difference for annual and perennial species, although it must be cautioned that this study did not take phylogenetic relatedness into account and habitats and probably level of pollination stochasticity were mixed. It is possible, of course, that life history is too coarse a proxy to adequately capture variation in breeding system. Nevertheless, evolutionary history could be relevant. Annual lineages are usually derived from perennial lineages (Friedman & Rubin, 2015) which will frequently be adapted for outcrossing. Strongly self-incompatible species suffer more pollen limitation than self-compatible species and especially when the latter are strongly autogamous (Larson & Barrett, 2000). In the transition from perenniality and outcrossing to the annual habitat coupled with selfing, in cases where autonomous selfing has been selected to provide reproductive assurance, the immediate ancestors of annuals, if they inhabited a stochastic pollination environment, are likely to have been subject to selection for higher ovule numbers as a response to a pollinator bottleneck. Seen in this light, larger ovule numbers in perennial species would not necessarily be expected. This possibility does not bode well for testing the relationship between breeding system and ovule oversupply in multispecies studies. In this context, phylogenetic studies on selected plant lineages that show variation in ovule number and breeding system and cover a wide range of pollination stochasticity would be useful. This approach could help in determining how similar numbers of ovules in annuals and perennials arise.

Our study is the first multispecies study on ovule bet-hedging to incorporate a measure of flower size. Flower size is a trait that comes under strong selection from pollinators (Ashman & Diefenderfer, 2001; Galen, 1996; Johnston, 1991; Parachnowitsch & Kessler, 2010; Totland, 2001; Wilson, Thomson, Stanton, & Rigney, 1994). For example, a number of studies have shown larger flowers to favour greater numbers of pollinator visits (e.g., Eckhart, 1991; Glaettli & Barrett, 2008; see Krizek & Anderson, 2013 for additional references) such that flower size might increase over elevation to assure visitation. However, ovule number it is also known to increase with flower size (cf, Davis, 1981; López et al., 1999; Wetzstein et al., 2013). As it turns out, we found no evidence for an increase in floral/pseudanthium biomass on our gradient, but we did find a tendency for more ovules as flower/pseudanthium biomass increased. Flowers/pseudanthia with larger numbers of ovules/florets, of course, will tend to have higher biomass. However, the increase in biomass in simple-flowered species due to additional ovules will be minimal given the negligent biomass of ovules, such that we can be

fairly confident that the relationship found in simple flowers primarily reflects the size of other flower parts. For pseudanthial species, an increase in floret number clearly will add significant biomass to a pseudanthium because all floret parts are involved. However, our conclusions for pseudanthial species are not altered, because pseudanthia for any given floral biomass over most of the size range represented in our study area have higher numbers of florets in the upper alpine. It is tempting to argue that the generally larger floret numbers at higher elevations in the central Chilean Andes are a consequence of selection for larger pseudanthia to attract scarcer pollinators. This is untenable on two counts: (a) pseudanthium size did not increase with elevation and (b) for equivalent numbers of florets, pseudanthia tend to be smaller in the high alpine. Overall, our results suggest that both pseudanthial and simple-flowered species in the upper alpine assign their resources differently, with greater emphasis on ovule production than at lower elevations.

The relationship between ovule oversupply and pollen limitation is a contentious issue. According to Burd et al. (2009) (see also Burd, 2016), ovule oversupply is likely to exacerbate pollen limitation independently of pollinator availability, given that ovule number per flower is expected to evolve well above the average number of successful fertilizations that occur in all flowers. Under these circumstances, where ovule number increases at higher elevations due to more stochastic pollination, levels of pollen limitation should increase markedly across the alpine gradient. The Burd et al. (2009) view has been recently challenged by Rosenheim et al. (2014), Rosenheim et al. (2016) on the grounds of pre- and postfertilization costs and a model of optimal plant reproductive allocations under stochastic pollen receipt. Knowledge on pollen limitation trends above tree line therefore become very relevant. Present knowledge, based on a relatively small and geographically biased sample of species, suggests around 50% of alpine species are pollen-limited García-Camacho and Totland (2009). However, very few studies have measured pollen limitation across the alpine gradient over the entire flowering season (Arroyo et al., 2017; Straka, & Starzomski, 2015; Trunschke & Stöcklin, 2017). Moreover, the alpine gradient involved are often fairly short leading to the risk of confounding effects of slope/aspect and local topography on elevational trends. It is therefore not possible at this time to say what the dominant elevational trend in pollen limitation above tree line is.

Finally, the question may be asked to what extent ovule bet-hedging can be expected in alpine floras in general. The answer here will depend on factors such as the relative richness and abundance of anthophilous insects, summer temperature, and plant breeding system. In some alpine species, flower longevity compensation seems to work quite well (Bingham & Orthner, 1998; Pickering, 1997), but in others, as in the Andes, it is not fail-safe (e.g., Wu et al., 2015). Insects appear to be sufficiently abundant to enable good seed set on some high nival species in the European Alps (Wagner et al., 2016). Nevertheless, it would not be surprisingly that the abundant visitation recorded in this study is recent and due to global warming which is strongly elevation-dependent (Falvey & Garreaud, 2009; Gobiet et al., 2014; Pepin et al., 2015) and is occurring at a faster rate in the northern hemisphere (Friedman, Hwang, Chiang, & Frierson, 2013). Effectively, warming could have changed the elevational distribution and abundance of anthophilous alpine insects in accordance with their different temperature optima (cf, Lefebvre, Villemant, Fontaine, & Daugeron, 2018). With respect to breeding system, while alpine floras contain many showy-flowered animal-pollinated species and levels of genetic diversity can be high (e.g., Ægisdóttir, Kuss, & Stöcklin, 2009; Arroyo & Squeo, 1990; Bingham & Ranker, 2000; Gaudeul, Taberlet, & Till-Bottraud, 2000; Gugerli, 1998; Kameyama & Kudo, 2009; Newport, 1989; Pojar, 1974; Rochefort & Peterson, 2001; Sobrevila, 1989; Trunschke & Stöcklin, 2017), the frequency of genetic self-incompatibility, where ovule bet-hedging is most expected, varies widely as seen in 44% in a subalpine meadow in Canada (Pojar, 1974), 30% in the harsh and windy Patagonian alpine (Arroyo & Squeo, 1990), and 3% in the Hengduan mountains in China (Peng et al., 2014). While the first two alpine areas compare favourably with the estimate of 40% self-incompatibility for flowering plants in general (Igic, Lande, & Kohn, 2008), the last falls well below. Therefore, on the grounds of breeding system, considerable variation in the propensity for ovule oversupply in alpine floras can be expected.

## 5 | CONCLUSIONS

Our work provides the first evidence for ovule oversupply in the alpine habitat and is the only study to date that has tested the ovule bet-hedging hypothesis at a broad community level. Our demonstration of parallel elevational increases in ovule/floret number in large suites of species belonging to two different floral categories that converge functionally in terms of pollinator attraction, irrespective of effects of pollination, and flowering period on ovule number, suggests strong selection on ovule number mediated by increasing unpredictable conditions for animal pollination over elevation in the alpine in the central Chilean Andes. As indicated earlier, we fully recognize that our work would have been greatly strengthened by some measure of flower-to-flower variation in potential mating success. The multispecies approach we used required sampling a very large number of species in order to adequately represent the alpine flora in two wide vegetation belts. Thus, this was out of the question. Site replication would also have been desirable. However, is it unlikely that our major conclusions would have changed in sampling a second alpine gradient. The alpine flora in the north–south trending Mediterranean-type climate Andes of central Chile where the tree line is naturally depressed, changes very little in species composition from one location to another. Meaningful site replication would entail moving to a different sector of the Andes such as the more northerly Altiplano. Splitting the alpine gradient into three, as we have done in previous studies long before the importance of phylogenetic relatedness became clear was also not possible because it would lead to considerable floristic overlap between elevational classes which

the Comparative Method cannot deal with. In any case, in order to determine how general our results are, additional studies along the Andes are in order. Finally, our work calls for all future studies on ovule oversupply to take flower size into account and for ecologists in general to make an effort to work with well-resolved phylogenetic trees at the tips.

## AUTHORS' CONTRIBUTIONS

M.T.K.A., F.P., P.J.-A., and D.P. conceived the ideas and designed the methodology; M.T.K.A., D.P., and M.F.F. collected and organized the field data; P.J.-A., P.V., F.P. undertook the DNA extractions and phylogenetic reconstruction: M.T.K.A., F.P., and D.P. analysed the data; M.T.K.A. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.f4j738r (Arroyo et al., 2018).

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## REFERENCES

Ægisdóttir, H. H., Kuss, P., & Stöcklin, J. (2009). Isolated populations of a rare alpine plant show high genetic diversity and considerable population differentiation. *Annals of Botany*, *104*(7), 1313–1322. https://doi.org/10.1093/aob/mcp242.

Ai, H. L., Zhou, W., Xu, K., Wang, H., & Li, D. Z. (2013). The reproductive strategy of a pollinator-limited Himalayan plant, *Incarvillea mairei* (Bignoniaceae). *BMC Plant Biology*, *13*(1), 195. https://doi.org/10.1186/1471-2229-13-195

Alcantara, S., & Lohmann, L. G. (2011). Contrasting phylogenetic signals and evolutionary rates in floral traits of Neotropical lianas. *Biological Journal of the Linnean Society*, *102*(2), 378–390. https://doi.org/10.1111/j.1095-8312.2010.01567.x.

APG IV (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, *181*(1), 1–20. https://doi.org/10.1111/boj.12385.

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 and visitation rates and population possibilities. *Plant Systematics and Evolution*, *149*, (1–2), 187–203. https://doi.org/10.1007/BF00983305.

Arroyo, M. T. K., Armesto, J. J., & Villagran, C. (1981). Plant phenological patterns in the high Andean cordillera of central Chile. *Journal of Ecology*, *69*(1), 205–223. https://doi.org/10.2307/2259826.

Arroyo, M. T. K., Dudley, L. S., Jespersen, G., Pacheco, D. A., & Cavieres, L. A. (2013). Temperature-driven flower longevity in a high-alpine species of *Oxalis* influences reproductive assurance. *New Phytologist*, *200*(4), 1260–1268. https://doi.org/10.1111/nph.12443.

Arroyo, M. T. K., Humaña, A., Dominguez, D., & Jespersen, G. (2012). Incomplete trimorphic incompatibility expression in *Oxalis compacta* Gill. ex Hook. et Arn. subsp *compacta* in the central Chilean Andes. *Gayana Botanica*, *69*(1), 88–99.

Arroyo, M. T. K., Muñoz, M. S., Henríquez, C., Till-Bottraud, I., & Pérez, 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-International Journal of Ecology*, *30*(2), 248–257. https://doi.org/10.1016/j.actao.2006.05.006

Arroyo, M. T. K., Pacheco, D. A., & Dudley, L. S. (2017). Functional role of long-lived flowers in preventing pollen limitation in a high elevation outcrossing species. *AoB Plants*, *9*(6), plx050. https://doi.org/10.1093/aobpla/plx050.

Arroyo, M. T. K., Pérez, F., Jara-Arancio, P., Pacheco, D., Vidal, P., & Flores, M. F. (2018). Data from: Ovule bet-hedging at high elevation in the South American Andes: Evidence from a phylogenetically-controlled multispecies study. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.f4j738r

Arroyo, M. T. K., Primack, R., & Armesto, J. (1982). Community studies in pollination ecology in the high temperate Andes of central Chile. 1. Pollination mechanisms and altitudinal variation. *American Journal of Botany*, *69*(1), 82–97. https://doi.org/10.2307/2442833.

Arroyo, M. T. K., & Squeo, F. (1990). Relationship between plant breeding systems and pollination. In S. Kawano (Ed.), *Biological approaches and evolutionary trends in plants* (pp. 205–227). London, UK: Academic Press.

Arroyo, M. T. K., & Uslar, P. (1993). Breeding systems in a temperate mediterranean-type climate montane sclerophyllous forest in central Chile. *Botanical Journal of the Linnean Society*, *111*(1), 83–102. https://doi.org/10.1006/bojl.1993.1008

Ashman, T. L., & Diefenderfer, C. (2001). Sex ratio represents a unique context for selection on attractive traits: Consequences for the evolution of sexual dimorphism. *The American Naturalist*, *157*(3), 334–347. https://doi.org/10.1086/319192

Aslan, C. E., Zavaleta, E. S., Tershy, B., & Croll, D. (2013). Mutualism disruption threatens global plant biodiversity: A systematic review. *PloS ONE*, *8*(6), e66993. https://doi.org/10.1371/journal.pone.0066993

Bergman, P., Molau, U., & Holmgren, B. (1996). Micrometeorological impacts on insect activity and plant reproductive success in an alpine environment, Swedish Lapland. *Arctic and Alpine Research*, *28*(2), 196–202. https://doi.org/10.2307/1551760.

Bingham, R. A., & Orthner, A. R. (1998). Efficient pollination of alpine plants. *Nature*, *391*, 238–239. https://doi.org/10.1038/34564

Bingham, R. A., & Ranker, T. A. (2000). Genetic diversity in alpine and foothill populations of *Campanula rotundifolia* (Campanulaceae). *International Journal of Plant Sciences*, *161*(3), 403–411. https://doi.org/10.1086/314272

Bond, W. (1994). Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical*

*Transactions of the Royal Society of London B*, *344*, 83–90. https://doi.org/10.1098/rstb.1994.0055

Burd, M. (1995). Ovule packaging in stochastic pollination and fertilization environments. *Evolution*, *49*(1), 100–109. https://doi.org/10.2307/2410296

Burd, M. (2016). Pollen limitation is common-should it be? (a comment on Rosenheim et al., "Parental optimism versus parental pessimism in plants: How common should we expect pollen limitation to be?"). *The American Naturalist*, *187*(3), 388–396. https://doi.org/10.1086/684848

Burd, M., Ashman, T.-L., Campbell, D. R., Dudash, M. R., Johnston, M. O., Knight, … Vamosi, J. C. (2009). Ovule number per flower in a world of unpredictable pollination. *American Journal of Botany*, *96*(6), 1159–1167. https://doi.org/10.3732/ajb.0800183

Carrasco, J. F., Casassa, G., & Quintana, J. (2005). Changes of the 0 degrees C isotherm and the equilibrium line altitude in central Chile during the last quarter of the 20th century. *Hydrological Sciences Journal-Journal Des Sciences Hydrologiques*, *50*(6), 933–948. https://doi.org/10.1623/hysj.2005.50.6.933.

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution*, *57*(12), 2742–2752. https://doi.org/10.1554/03-215.

Cavieres, L. A., & Arroyo, M. T. K. (1999). Tasa de enfriamiento adiabático del aire en el Valle del Río Molina, Provincia de Santiago, Chile Central (33°S). *Revista Geográfica Terra Australis*, *44*, 79–86.

Cavieres, L. A., Peñaloza, A., & Arroyo, M. T. K. (2000). Altitudinal vegetation belts in the high-Andes of central Chile (33 degrees S). *Revista Chilena de Historia Natural*, *73*(2), 331–344. https://doi.org/10.4067/S0716-078X2000000200008

Chalcoff, V. R., & Aizen, M. A. (2016). Pollination unpredictability and ovule number in a South-Andean Proteaceae along a rainfall gradient. *Australian Journal of Botany*, *64*(1), 8–14. https://doi.org/10.1071/BT15016

Clauss, M. J., & Venable, D. L. (2000). Seed germination in desert annuals: An empirical test of adaptive bet hedging. *The American Naturalist*, *155*(2), 168–186. https://doi.org/10.1086/303314

Corbet, S. A. (1990). Pollination and the weather. *Israel Journal of Botany*, *39*(1–2), 13–30.

Davis, M. A. (1981). The effect of pollinators, predators, and energy constraints on the floral ecology and evolution of *Trillium erectum*. *Oecologia*, *48*(3), 400–406. https://doi.org/10.1007/BF00346501

Duan, Y. W., Zhang, T. F., & Liu, J. Q. (2007). Interannual fluctuations in floral longevity, pollinator visitation and pollination limitation of an alpine plant (*Gentiana straminea* Maxim., Gentianaceae). at two altitudes in the Qinghai-Tibetan Plateau. *Plant Systematics and Evolution*, *267*(1), 255–265. https://doi.org/10.1007/s00606–007–0553-y

Dudley, L. S., Arroyo, M. T. K., & Fernández-Murillo, M. P. (2018). Physiological and fitness response of flowers to temperature and water augmentation in a high Andean geophyte. *Environmental and Experimental Botany*, *150*, 1–8. https://doi.org/10.1016/j.envexpbot.2018.02.015

Eckhart, V. M. (1991). The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology*, *5*(4), 370–384. https://doi.org/10.1007/BF02214154

Eriksen, B., Molau, U., & Svensson, M. (1993). Reproductive strategies in 2 Arctic *Pedicularis* species (Scrophulariaceae). *Ecography*, *16*(2), 154–166. https://doi.org/10.1111/j.1600-0587.1993.tb00067.x

Evans, M. E. K., Ferriere, R., Kane, M. J., & Venables, D. L. (2007). Bet hedging via seed banking in desert evening primroses (*Oenothera*, Onagraceae): Demographic evidence from natural populations. *The American Naturalist*, *169*(2), 184–194. https://doi.org/10.1086/510599

Explorador climático. Centro del Ciencia del Clima y Resiliencia (CR)2, Santiago, Chile. Retrieved from https://explorador.cr2.cl/

Falvey, M., & Garreaud, R. D. (2009). Regional cooling in a warming world: Recent temperature trends in the southeast Pacific and along the west coast of subtropical South America (1979–2006). *Journal of Geophysical Research-Atmospheres*, *114*, 1979–2006. https://doi.org/10.1029/2008jd010519

Friedman, A. R., Hwang, Y. T., Chiang, J. C. H., & Frierson, D. M. W. (2013). Interhemispheric temperature asymmetry over the twentieth century and in future projections. *Journal of Climate*, *26*(15), 5419–5433. https://doi.org/10.1175/jcli-d-12-00525.1

Friedman, J., & Rubin, M. J. (2015). All in good time: Understanding annual and perennial strategies in plants. *American Journal of Botany*, *102*(4), 497–499. https://doi.org/10.3732/ajb.1500062

Fulkerson, J. R., Whittall, J. B., & Carlson, M. L. (2012). Reproductive ecology and severe pollen limitation in the polychromic tundra plant, *Parrya nudicaulis* (Brassicaceae). *PLoS ONE*, *7*(3), e32790. https://doi.org/10.1371/journal.pone.0032790

Galen, C. (1996). Rates of floral evolution: Adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution*, *50*(1), 120–125. https://doi.org/10.1111/j.1558-5646.1996.tb04478.x

García-Camacho, R., & Totland, Ø. (2009). Pollen limitation in the alpine: A meta-analysis. *Arctic Antarctic and Alpine Research*, *41*(1), 103–111. https://doi.org/10.1657/1523-0430-41.1.103

Gaudeul, M., Taberlet, P., & Till-Bottraud, I. (2000). Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology*, *9*(10), 1625–1637. https://doi.org/10.1046/j.1365-294x.2000.01063.x

Glaettli, M., & Barrett, S. C. H. (2008). Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae). *New Phytologist*, *179*(4), 1193–1201. https://doi.org/10.1111/j.1469-8137.2008.02532.x

Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., & Stoffel, M. (2014). 21st century climate change in the European Alps-A review. *Science of the Total Environment*, *493*, 1138–1151. https://doi.org/10.1016/j.scitotenv.2013.07.050

Gómez, J. M., Abdelaziz, M., Lorite, J., Jesus Munoz-Pajares, A., & Perfectti, F. (2010). Changes in pollinator fauna cause spatial variation in pollen limitation. *Journal of Ecology*, *98*(5), 1243–1252. https://doi.org/10.1111/j.1365-2745.2010.01691.x

Gugerli, F. (1998). Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia*, *114*(1), 60–66. https://doi.org/10.1007/s004420050420

Herrera, C. M. (1987). Components of pollinator quality – Comparative-analysis of a diverse insect assemblage. *Oikos*, *50*(1), 79–90. https://doi.org/10.2307/3565403

Hirao, A. S., Kameyama, Y., Ohara, M., Isagi, Y., & Kudo, G. (2006). Seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub *Rhododendron aureum* (Ericaceae). *Molecular Ecology*, *15*(4), 1165–1173. https://doi.org/10.1111/j.1365-294X.2006.02853.x

Hocking, B. (1968). Insect-flower associations in the high Arctic with special reference to nectar. *Oikos*, *19*(2), 359–387. https://doi.org/10.2307/3565022

Igic, B., Lande, R., & Kohn, J. R. (2008). Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences*, *169*(1), 93–104. https://doi.org/10.1086/523362

Johnston, M. O. (1991). Natural-selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution*, *45*(6), 1468–1479. https://doi.org/10.2307/2409893

Jones, K. N. (1997). Analysis of pollinator foraging: Tests for non-random behaviour. *Functional Ecology*, *11*(2), 255–259. https://doi.org/10.1046/j.1365-2435.1997.00080.x

Judd, W. S., Campbell, C. S., Kellog, E. A., Stevens, P. F., & Donoghue, M. J. (2008). *Plant systematics: A phylogenetic approach*. Sunderland, MA: Sinauer Associates, Inc.

Kameyama, Y., & Kudo, G. (2009). Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: Effects of pollinators and self-incompatibility. *Annals of Botany*, 103(9), 1385–1394. https://doi.org/10.1093/aob/mcp037

Kasagi, T., & Kudo, G. (2003). Variations in bumble bee preference and pollen limitation among neighboring populations: Comparisons between *Phyllodoce caerulea* and *Phyllodoce aleutica* (Ericaceae) along snowmelt gradients. *American Journal of Botany*, 90(9), 1321–1327. https://doi.org/10.3732/ajb.90.9.1321

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., … Ashman, T. L. (2005). Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology Evolution and Systematics*, 36, 467–497. https://doi.org/10.1146/annurev.ecolsys.36.102403.115320

Körner, C. (2003). *Alpine plant life. Functional plant ecology of high mountain ecosystems* (2nd ed). Berlin, Germany; New York, NY: Springer-Verlag.

Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation: Small bees exploit the plant-pollinator mutualism. *Proceedings of the Royal Society B-Biological Sciences*, 285(1880), 20180635. https://doi.org/10.1098/rspb.2018.0635

Krizek, B. A., & Anderson, J. T. (2013). Control of flower size. *Journal of Experimental Botany*, 64(6), 1427–1437. https://doi.org/10.1093/jxb/ert025

Kudo, G. (1993). Relationship between flowering time and fruit set of the entomophilous alpine shrub, *Rhododendron aureum* (Ericaceae), inhabiting snow patches. *American Journal of Botany*, 80(11), 1300–1304. https://doi.org/10.1111/j.1365-294X.2006.02853.x

Kudo, G., & Hirao, A. S. (2006). Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Population Ecology*, 48(1), 49–58. https://doi.org/10.1007/s10144-005-0242-z

Kudo, G., Hirao, A. S., & Kawai, Y. (2011). Pollination efficiency of bumblebee queens and workers in the alpine shrub *Rhododendron aureum*. *International Journal of Plant Sciences*, 172(1), 70–77. https://doi.org/10.1086/657282

Ladd, P. G., & 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), 2–10. https://doi.org/10.1111/j.1442-1984.2009.00234.x

Ladinig, U., Hacker, J., Neuner, G., & Wagner, J. (2013). How endangered is sexual reproduction of high-mountain plants by summer frosts? Frost resistance, frequency of frost events and risk assessment. *Oecologia*, 171(3), 743–760. https://doi.org/10.1007/s00442-012-2581-8

Ladinig, U., & Wagner, J. (2007). Timing of sexual reproduction and reproductive success in the high-mountain plant *Saxifraga bryoides* L. *Plant Biology*, 9(6), 683–693. https://doi.org/10.1055/s-2007-965081

Larson, B. M. H., & Barrett, S. C. H. (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, 69(4), 503–520. https://doi.org/10.1111/j.1095-8312.2000.tb01221.x

Lázaro, A., Hegland, S. J., & Totland, O. (2008). The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia*, 157(2), 249–257. https://doi.org/10.1007/s00442-008-1066-2

Lázaro, A., Lundgren, R., & Totland, O. (2015). Pollen limitation, species' floral traits and pollinator visitation: Different relationships in contrasting communities. *Oikos*, 124(2), 174–186. https://doi.org/10.1111/oik.01525

Lefebvre, V., Villemant, C., Fontaine, C., & Daugeron, C. (2018). Altitudinal, temporal and trophic partitioning of flower-visitors in alpine communities. *Scientific Reports*, 8, 1–12. https://doi.org/10.1038/s41598-018-23210-y

López, J., Rodriguez-Riano, T., Ortega-Olivencia, A., Devesa, J. A., & Ruiz, T. (1999). Pollination mechanisms and pollen-ovule ratios in some Genisteae (Fabaceae) from Southwestern Europe. *Plant Systematics and Evolution*, 216(1–2), 23–47. https://doi.org/10.1007/BF0098509

Lundemo, S., & Totland, O. (2007). Within-population spatial variation in pollinator visitation rates, pollen limitation on seed set, and flower longevity in an alpine species. *Acta Oecologica-International Journal of Ecology*, 32(3), 262–268. https://doi.org/10.1016/j.actao.2007.05.007

Maad, J., Armbruster, W. S., & Fenster, C. B. (2013). Floral size variation in *Campanula rotundifolia* (Campanulaceae) along altitudinal gradients: Patterns and possible selective mechanisms. *Nordic Journal of Botany*, 31(3), 361–371. https://doi.org/10.1111/j.1756-1051.2013.01766.x

Márten-Rodriguez, S., & Fenster, C. B. (2010). Pollen limitation and reproductive assurance in Antillean Gesnerieae: A specialists vs. generalist comparison. *Ecology*, 91(1), 155–165. https://doi.org/10.1890/08-2115.1

Mayfield, M. M., Waser, N. M., & Price, M. V. (2001). Exploring the 'most effective pollinator principle' with complex flowers: Bumblebees and *Ipomopsis aggregata*. *Annals of Botany*, 88(4), 591–596. https://doi.org/10.1006/anbo.2001.1500

McCall, C., & Primack, R. B. (1992). Influence of flower characteristics, weather, time of day, and season on insect visitation rates in 3 plant-communities. *American Journal of Botany*, 79(4), 434–442. https://doi.org/10.2307/2445156

Medan, D. (2003). Reproductive biology of the Andean shrub *Discaria nana* (Rhamnaceae). *Plant Biology*, 5(1), 94–101. https://doi.org/10.1055/s-2003-37980

Medan, D., Montaldo, N. H., Devoto, M., Mantese, A., Vasellati, V., & Bartoloni, N. H. (2002). Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic Antarctic and Alpine Research*, 34(3), 233–241. https://doi.org/10.2307/1552480

Medan, D., Zarlavsky, G., & Bartoloni, N. J. (2013). Plant reproduction in the high-Andean Puna: *Kentrothamnus weddellianus* (Rhamnaceae: Colletieae). *Plant Systematics and Evolution*, 299(5), 841–851. https://doi.org/10.1007/s00606-013-0766-1

Mitchell, R. J., Irwin, R. E., Flanagan, R. J., & Karron, J. D. (2009). Ecology and evolution of plant-pollinator interactions. *Annals of Botany*, 103(9), 1355–1363. https://doi.org/10.1093/aob/mcp122

Mizunaga, Y., & Kudo, G. (2017). A linkage between flowering phenology and fruit-set success of alpine plant communities with reference to the seasonality and pollination effectiveness of bees and flies. *Oecologia*, 185(3), 453–464. https://doi.org/10.1007/s00442-017-3946-9

Mort, M. E., Archibald, J. K., Randle, C. P., Levsen, N. D., O'Leary, T. R., Topalov, K., Crawford, D. J. (2007). Inferring phylogeny at low taxonomic levels: Utility of rapidly evolving cpDNA and nuclear ITS loci. *American Journal of Botany*, 94(2), 173–183. https://doi.org/10.3732/ajb.94.2.173

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(4), 608–613. https://doi.org/10.1657/1523-0430(2006).38[608:plasvo]2.0.co;2

Muñoz, A. A., & Cavieres, L. A. (2008). The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *Journal of Ecology*, 96(3), 459–467. https://doi.org/10.1111/j.1365-2745.2008.01361.x

Newport, M. E. A. (1989). A test for proximity-dependent outcrossing in the alpine skypilot, *Polemonium viscosum*. *Evolution*, 43(5), 1110–1113. https://doi.org/10.1111/j.1558-5646.1989.tb02555.x

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Orme, D. (2018). *The caper package: Comparative analysis of phylogenetics and evolution in R*. Retrieved from https://cran.r-project.org/web/packages/caper/vignettes/caper.pdf

Pacheco, D. A., Dudley, L. S., Cabezas, J., Cavieres, L. A., & Arroyo, M. T. K. (2016). Plastic responses contribute to explaining altitudinal and temporal variation in potential flower longevity in high Andean *Rhodolirion montanum*. *PloS ONE*, *11*(11), e0166350. https://doi.org/10.1371/journal.pone.0166350

Parachnowitsch, A. L., & Kessler, A. (2010). Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist*, *188*(2), 393–402. https://doi.org/10.1111/j.1469-8137.2010.03410.x

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. Retrieved from https://cran.r-project.org/web/packages/ape/ape.pdf. 10.1093/bioinformatics/btg412

Peng, D.-L., Ou, X.-K., Xu, B., Zhang, Z.-Q., Niu, Y., Li, Z.-M., & Sun, H. (2014). Plant sexual systems correlated with morphological traits: Reflecting reproductive strategies of alpine plants. *Journal of Systematics and Evolution*, *52*(3), 368–377. https://doi.org/10.1111/jse.12046

Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., ... Yang, D.; Mountain Research Initiative EDW Working Group (2015). Elevation-dependent warming in mountain regions of the world. *Nature Climate Change*, *5*(5), 424–430. https://doi.org/10.1038/nclimate2563

Pérez, F., Arroyo, M. T. K., & Armesto, J. J. (2009). Evolution of autonomous selfing accompanies increased specialization in the pollination system of *Schizanthus* (Solanaceae). *American Journal of Botany*, *96*(6), 1168–1176. https://doi.org/10.3732/ajb.0800306

Philippi, T., & Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends in Ecology and Evolution*, *4*(2), 41–44. https://doi.org/10.1016/0169-5347(89).90138-9

Pickering, C. M. (1997). Breeding systems of Australian *Ranunculus* in the alpine region. *Nordic Journal of Botany*, *17*(6), 613–620. https://doi.org/10.1111/j.1756-1051.1997.tb00357.x

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D.; R Core Team (2018). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137, Retrieved from https://CRAN.R-project.org/package=nlme.

Piper, F. I., Vinegla, B., Linares, J. C., Camarero, J. J., Cavieres, L. A., & Fajardo, A. (2016). Mediterranean and temperate treelines are controlled by different environmental drivers. *Journal of Ecology*, *104*(3), 691–702. https://doi.org/10.1111/1365-2745.12555

Pojar, J. (1974). Reproductive dynamics of four plant communities of southwestern British Columbia. *Canadian Journal of Botany*, *52*(8), 1819–1834. https://doi.org/10.1139/b74-234

Pozner, R., Zanotti, C., & Johnson, L. A. (2012). Evolutionary origin of the Asteraceae capitulum: Insights from Calyceraceae. *American Journal of Botany*, *99*(1), 1–13. https://doi.org/10.3732/ajb.1100256

Primack, R. B., & Inouye, D. W. (1993). Factors affecting pollinator visitation rates – A biogeographic comparison. *Current Science*, *65*(3), 257–262.

Ramos-Jiliberto, R., Dominguez, D., Espinoza, C., Lopez, G., Valdovinos, F. S., Bustamante, R. O., & Medel, R. (2010). Topological change of Andean plant-pollinator networks along an altitudinal gradient. *Ecological Complexity*, *7*(1), 86–90. https://doi.org/10.1016/j.ecocom.2009.06.001

Razanajatovo, M., Maurel, N., Dawson, W., Essl, F., Kreft, H., Pergl, J., van Kleunen, M. (2016). Plants capable of selfing are more likely to become naturalized. *Nature Communications*, *7*, 13313. https://doi.org/10.1038/ncomms13313

Rochefort, R. M., & Peterson, D. L. (2001). Genetic and morphologic variation in *Phyllodoce empetriformis* and *Phyllodoce glanduliflora* (Ericaceae) in Mount Rainier, National Park, Washington. *Canadian Journal of Botany-Revue Canadienne De Botanique*, *79*(2), 179–191. https://doi.org/10.1139/b00-147

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Rosenheim, J. A., Schreiber, S. J., & Williams, N. M. (2016). Does an 'oversupply' of ovules cause pollen limitation? *New Phytologist*, *210*(1), 324–332. https://doi.org/10.1111/nph.13750

Rosenheim, J. A., Williams, N. M., & Schreiber, S. J. (2014). Parental optimism versus parental pessimism in plants: How common should we expect pollen limitation to be? *The American Naturalist*, *184*(1), 75–90. https://doi.org/10.1086/676503

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Saunders, M. E., Peisley, R. K., Rader, R., & Luck, G. W. (2016). Pollinators, pests, and predators: Recognizing ecological trade-offs in agroecosystems. *Ambio*, *45*(1), 4–14. https://doi.org/10.1007/s13280-015-0696-y

Schreiber, S. J., Rosenheim, J. A., Williams, N. W., & Harder, L. D. (2015). Evolutionary and ecological consequences of multiscale variation in pollen receipt for seed production. *The American Naturalist*, *185*(1), E14–E29. https://doi.org/10.1086/678982

Seguí, J., Lázaro, A., Traveset, A., Salgado-Luarte, C., & Gianoli, E. (2018). Phenotypic and reproductive responses of an Andean violet to environmental variation across an elevational gradient. *Alpine Botany*, *128*, 59–69. https://doi.org/10.1007/s00035-017-0195-9

Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B-Biological Sciences*, *278*(1712), 1601–1609. https://doi.org/10.1098/rspb.2011.0176

Snell, R., & Aarssen, L. W. (2005). Life history traits in selfing versus outcrossing annuals: Exploring the 'time-limitation' hypothesis for the fitness benefit of self-pollination. *BMC Ecology*, *5*, 2. https://doi.org/10.1186/1472-6785-5-2

Sobrevila, C. (1989). Effects of pollen donors on seed formation in *Espeletia schultzii* (Compositae) populations at different altitudes. *Plant Systematics and Evolution*, *166*(1–2), 45–67. https://doi.org/10.1007/BF00937875

Squeo, F. A., Cepeda, J., Olivares, N. C., & Arroyo, M. T. K. (2006). Interacciones ecológicas en la alta montaña del Valle del Elqui. In J. Cepeda (Ed.), *Geoecología de los Andes Desérticos: La Alta Montaña del Valle del Elqui* (pp. 69–103). La Serena, Chile: Ediciones Universidad de La Serena.

Steinacher, G., & Wagner, J. (2010). Flower longevity and duration of pistil receptivity in high mountain plants. *Flora*, *205*(6), 376–387. https://doi.org/10.1016/j.flora.2009.12.012

Straka, J. R., & Starzomski, B. M. (2015). Fruitful factors: What limits seed production of flowering plants in the alpine? *Oecologia*, *178*(1), 249–260. https://doi.org/10.1007/s00442-014-3169-2

Tiusanen, M., Hebert, P. D. N., Schmidt, N. M., & Roslin, T. (2016). One fly to rule them all—Muscid flies are the key pollinators in the Arctic. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1839), 20161271. https://doi.org/10.1098/rspb.2016.1271

Torres-Diaz, C., Cavieres, L. A., Muñoz-Ramírez, C., & Arroyo, M. T. K. (2007). Consequences of microclimate variation on insect pollinator visitation in two species of *Chaetanthera* (Asteraceae) in the central Chilean Andes. *Revista Chilena de Historia Natural*, *80*(4), 455–468. https://doi.org/10.4067/S0716-078X2007000400007

Torres-Díaz, C., Gómez-González, S., Stotz, G. C., Torres-Morales, P., Paredes, B., Pérez-Millaqueo, M., & Gianoli, E. (2011). Extremely long-lived stigmas allow extended cross-pollination opportunities in a high Andean Plant. *PLoS ONE*, *6*(5), e19497. https://doi.org/10.1371/journal.pone.0019497

Totland, Ø. (1994). Influence of climate, time of day and season, and flower density on insect flower visitation in alpine Norway. *Arctic and Alpine Research*, *26*(1), 66–71. https://doi.org/10.2307/1551879

Totland, Ø. (1997). Limitations on reproduction in alpine *Ranunculus acris*. *Canadian Journal of Botany-Revue Canadienne De Botanique*, *75*(1), 137–144. https://doi.org/10.1139/b97-016

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, *82*(8), 2233–2244. https://doi.org/10.2307/2680228

Trunschke, J., & Stöcklin, J. (2017). Plasticity of flower longevity in alpine plants is increased in populations from high elevation compared to low elevation populations. *Alpine Botany*, *127*(1), 41–51. https://doi.org/10.1007/s00035-016-0176-4

Tur, C., Sáez, A., Traveset, A., & Aizen, M. A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: Evidence of widespread facilitation in south Andean plant communities. *Ecology Letters*, *19*(5), 576–586. https://doi.org/10.1111/ele.12594

Utelli, A. B., & Roy, B. A. (2000). Pollinator abundance and behavior on *Aconitum lycoctonum* (Ranunculaceae): An analysis of the quantity and quality components of pollination. *Oikos*, *89*(3), 461–470. https://doi.org/10.1034/j.1600-0706.2000.890305.x

Vandelook, F., Verdu, M., & Honnay, O. (2012). The role of seed traits in determining the phylogenetic structure of temperate plant communities. *Annals of Botany*, *110*(3), 629–636. Retrieved from https://cran.rproject.org/web/packages/MASS/index.html. 10.1093/aob/mcs121

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). New York, NY: Springer.

Viale, M., & Garreaud, R. (2014). Summer precipitation events over the western slope of the subtropical Andes. *Monthly Weather Review*, *142*(3), 1074–1092. https://doi.org/10.1175/mwr-d-13-00259.1

Wagner, J., Lechleitner, M., & Hosp, D. (2016). Pollen limitation is not the rule in nival plants: A study from the European Central Alps. *American Journal of Botany*, *103*(3), 375–387. https://doi.org/10.3732/ajb.1500214

Waites, A. R., & Ågren, J. (2004). Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology*, *92*(3), 512–526. https://doi.org/10.1111/j.0022-0477.2004.00893.x

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Weberling, F. (1998). Monotele und polytele Synfloreszenzen. In W.Troll (Ed.), *Die Infloreszenzen, Typologie und Stellung in Aufbau des Vegetationskörpers II/2*. Jena, Germany: G. Fischer. https://doi.org/10.1111/j.1756-1051.1999.tb01114.x

Wetzstein, H. Y., Yi, W. G., Porter, J. A., & Ravid, N. (2013). Flower position and size impact ovule number per flower, fruitset, and fruit size in pomegranate. *Journal of the American Society for Horticultural Science*, *138*(3), 159–166. Retrieved from https://journal.ashspublications.org/content/138/3/159.full.pdf

Wiens, D. (1984). Ovule survivorship, brood size, life-history, breeding systems, and reproductive success in plants. *Oecologia*, *64*(1), 47–53. https://doi.org/10.1007/bf00377542

Wiklund, C., Eriksson, T., & Lundberg, H. (1979). Wood white butterfly *Leptidea sinapis* and its nectar plants – A case of mutualism or parasitism. *Oikos*, *33*(3), 358–362. https://doi.org/10.2307/3544323

Willis, K. J. (Ed.) (2016). *State of the world´s plants*. London, UK: Royal Botanic Gardens, Kew. Retrieved from https://stateoftheworldsplants.com/2016/

Wilson, P., Thomson, J. D., Stanton, M. L., & Rigney, L. P. (1994). Beyond floral Batemania – Gender biases in selection for pollination success. *The American Naturalist*, *143*(2), 283–296. https://doi.org/10.1086/285604

Wolowski, M., Ashman, T. L., & Freitas, L. (2014). Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic Forest of Brazil. *Plos One*, *9*(2), e89498. https://doi.org/10.1371/journal.pone.0089498

Wu, Y., Liu, Y.-R., Peng, H., Yang, Y., Liu, G.-L., Cao, G.-X., & Zhang, Q. (2015). Pollination ecology of alpine herb *Meconopsis integrifolia* at different altitudes. *Chinese Journal of Plant Ecology*, *39*(1), 1–13. https://doi.org/10.17521/cjpe.2015.0001

Yang, J., Ci, X. Q., Lu, M. M., Zhang, G. C., Cao, M., Li, J., & Lin, L. X. (2014). Functional traits of tree species with phylogenetic signal co-vary with environmental niches in two large forest dynamics plots. *Journal of Plant Ecology*, *7*(2), 115–125. https://doi.org/10.1093/jpe/rtt070

## SUPPORTING INFORMATION

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