

## POLLEN LIMITATION AND REPRODUCTIVE ASSURANCE IN THE FLORA OF THE COASTAL ATACAMA DESERT

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Pollen limitation (PL) in plant populations is supposed to be particularly strong in variable pollinator environments. Here, we examined the extent of PL in the coastal Atacama Desert, where low and unpredictable rainfall drives large interannual variation in plant cover and pollinator abundances. We estimated PL levels and the capacity for autonomous selfing (autofertility) in 16 annual and perennial species. In addition, we compared fruit set of emasculated and intact flowers to test whether selfing provides reproductive assurance. We also examined the relationships between autofertility and life forms, between PL and autofertility, and between PL and flower size. We found a low level of PL (mean = 0.28) and a high incidence of autofertility, which was negatively correlated with PL and closely related to life form. Our results suggest that the low PL levels in autonomous-selfing species result from the reproductive assurance provided by selfing. Considering nonautonomous species only, the mean PL (0.44) was similar to that reported for other habitats. We detected no association between PL and flower size. In summary, high rates of PL were not a general pattern among plants in the Atacama Desert. The low levels of PL can be attributed to the high incidence of autofertility.

**Keywords:** Atacama Desert, autonomous selfing, independent-contrast analysis, pollen limitation, reproductive assurance.

### Introduction

In flowering plants, female reproductive success is often constrained by the supply of pollen, a phenomenon known as pollen limitation (PL hereafter; Burd 1994; Larson and Barrett 2000; Ashman et al. 2004). In general, 60% of angiosperms exhibit significant levels of PL (Burd 1994; Larson and Barrett 2000; Knight et al. 2005), and this high PL incidence has been associated with the stochastic nature of pollination. PL may represent an evolutionarily stable strategy to cope with pollinator unpredictability (Burd 1995). When plant populations experience uncertain and highly variable acquisition of pollen, natural selection may favor the production of an excess number of ovules or flowers relative to the average pollen capture, as a “bet-hedging” mechanism to maximize reproductive success during years of abundant pollination (Burd 1995; Ashman et al. 2004). Recent evidence for the importance of pollination unpredictability on the evolution of ovule packaging was provided by Burd et al. (2009), who found a strong positive correlation between the number of ovules produced per flower and the variation in pollen receipt or seed set. Thus, PL should be particularly strong in plant populations occurring in habitats where pollinator availability is highly fluctuating over many years.

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Variable pollination environments, however, can also favor the evolution of several strategies to ameliorate the levels of PL. Theory predicts that pollinator variability may intensify the selection for autonomous selfing (i.e., the ability of flowers to self-fertilize in the absence of external vectors; Morgan and Wilson 2005), which provides reproductive assurance when pollinators fail (Baker 1955; Stebbins 1974; Lloyd 1992; Goodwillie et al. 2005). As a result of reproductive assurance, autonomous-selfing species are predicted to be less pollen limited than non-autonomous-selfing species (Larson and Barrett 2000). Empirical evidence for increasing seed set due to autonomous selfing, under variable pollination environments, is available for few species (Kalisz and Vogler 2003; Pérez et al. 2009). For example, Kalisz and Vogler (2003) compared the seed set of emasculated and intact flowers of natural populations of *Collinsia verna* over several years and showed that autonomous selfing increased seed set over vector-mediated pollination during years of pollinator failure. The consequences of variable pollination environments on selfing evolution, however, depend on species' life forms (Morgan et al. 1997). In general, a perennial life form makes the evolution of selfing more difficult, possibly because the reproductive assurance provided by selfing is less significant in long-lived plants than in annuals and because the disadvantages of selfing (inbreeding depression and seed discount) are more pronounced in perennials (Petit and Hampe 2006).

Another way for plants to escape the consequences of pollinator variability and PL is the evolution of floral traits that

increase the chances of vector-mediated pollination. A high investment in floral display or nectar production can enhance flower attractiveness and the probability of pollination (Billings and Mooney 1968; Fabbro and Körner 2004). Accordingly, the degree of PL is expected to be lower in plants with larger flowers (Haig and Westoby 1988). However, a comparative study across multiple species did not detect a relationship between PL and corolla size (Knight et al. 2005).

The relation between the variability of pollinator environments and the extent of PL has been scarcely examined in natural populations, with contrasting results. For example, Stone and Jenkins (2007) showed that PL was lower in lowland populations of *Witheringia solanacea* than in populations growing at the altitudinal margin of the species distribution, where climate was less predictable, thus supporting the bet-hedging hypothesis. In contrast, García-Camacho and Totland (2009) did not find significant differences in PL between alpine and lowland species, pointing out the evolution of “compensatory” mechanisms to mitigate the low and fluctuating availability of pollinators in alpine environments. Clearly, more experimental studies of species in extreme habitats that consider a diversity of life forms are needed to obtain a more general picture of PL.

In this study, we assess the extent of PL in the flora of the coastal Atacama Desert of north-central Chile, where short and infrequent rainfall events result in periodically enhanced soil moisture that regulates plant production and phenological patterns (Vidiella and Armesto 1989). This region is characterized by 2–5 yr of drought, followed by short and variable rainfall events. A major proportion of the biota remains dormant during long periods of drought. After rainfall events, there is an explosive increase in plant cover, composed mainly of short-lived annuals, bulbous geophytes, and summer-dormant shrubs (Vidiella and Armesto 1989). The fast growth rates and abundant flowering after rainfall, a phenomenon known locally as the “blooming of the desert,” represent a sharp increase in the resources available to pollinators (Vidiella and Armesto 1989; Toro et al. 1996).

We assessed the magnitude of PL, using supplemental hand-pollination experiments, and the capacity for autonomous selfing (autofertility sensu Schoen and Lloyd 1992) of 16 plant species from the coastal Atacama Desert, including shrubs, annuals, and perennial herbs. We also conducted emasculation experiments in those plant species capable of autonomous selfing to assess whether sufficient pollen is deposited by pollinators for full fruit set (pollinator failure sensu Kalisz and Vogler 2003) and to estimate the contribution of autonomous selfing to natural fruit set (reproductive assurance sensu Schoen and Lloyd 1992). Finally, we used comparative approaches that account for phylogeny (Felsenstein 1985) to examine the relationships between autofertility and life forms, between PL and autofertility, and between PL and flower size. Using this approach, we acknowledge the fact that species are not independent entities and have a common history. These procedures allowed us to address the following specific questions: (1) Is the fruit set of Atacama Desert species strongly limited by pollen? (2) Are both annual and perennial desert species capable of autonomous selfing? (3) Does autonomous selfing provide reproductive assurance when pollinators fail? (4) Does PL decrease with autofertility

as a result of reproductive assurance? (5) Are the species with smaller floral displays subject to more PL than species with larger floral displays? Because of the high interannual variation in rainfall, plant cover, and pollinator abundance in the coastal Atacama Desert, we expected that desert plants would show strong PL. Alternatively, desert species may have acquired the capacity for autonomous selfing (in the case of annual plants) or a larger flower size (in the case of perennials) to increase the likelihood of successful pollination, in which case factors other than pollen availability would constrain fruit production.

## Material and Methods

### Study Site and Species

The study was conducted in Llanos del Challe National Park (lat 28°13'S, long 71°04'W), a coastal site located in the southern part of the Atacama Desert (Chile). The climate is arid, with a Mediterranean influence (Di Castri and Hajek 1976). The mean annual precipitation is 40 mm, the mean annual relative humidity is about 70%–80%, and the mean annual temperature ranges from 14.7° to 16.1°C. Precipitation is highly variable within and among years. The lowest and highest annual precipitation rates reported are 0 and 120 mm, respectively. High-precipitation years are usually associated with El Niño–Southern Oscillation (ENSO) events (Holmgren et al. 2006).

Controlled pollination experiments were carried out during October 2008 on 16 hermaphrodite and entomophilous plant species, the majority of them endemic to the coastal Atacama Desert (table 1). Species were selected so as to include shrubs, perennial herbs, and annual herbs. Handling possibilities, the presence of sufficient individuals of a given species in the area, and phenology also influenced selection. Only species initiating flowering or at peak flowering were included. Flower size of each species was measured from frontal photos of five flowers taken from five individuals per species. Corolla diameter or the diameter of inflorescence head (in the case of *Quinchamalium chilense*) was used as a measurement of flower size.

### Pollination Experiments

Forty plants per species were randomly selected. Three flower buds were randomly chosen on each plant and tagged before applying one of the following treatments: (H) supplemental hand-pollinated flowers received additional pollen from at least three plants of the same species located 20 m away; (E) emasculated flowers had their anthers removed at the bud stage and were left exposed to natural pollination; (N) nonmanipulated control flowers were exposed to natural pollination. These three treatments could not be carried out on the same individual plants in the case of *Rodophiala bag-noldii* because not enough buds were available on a single plant. Another 40 plants per species were randomly selected to estimate the capacity for autonomous selfing. One flower bud for each plant was bagged with a fine, light white fabric and isolated from the intervention of pollinators. The percentage of flowers that set fruit was estimated for each treat-

**Table 1**  
**Coastal Atacama Desert Species Examined in This Study**

	Family	Life form	Corolla size mean $\pm$ SE	Distribution
<i>Adesmia argyrophylla</i> Phil. F	Fabaceae	Shrub	.69 $\pm$ .01	Chile: III
<i>Alstroemeria philippi</i> Baker	Alstroemeriaceae	Perennial herb	7.36 $\pm$ .63	Chile: III
<i>Alstroemeria werdermannii</i> Bayer	Alstroemeriaceae	Perennial herb	7.27 $\pm$ .68	Chile: III
<i>Balsisia peduncularis</i> (Lindl.) D. Don	Ledocarpaceae	Shrub	3.98 $\pm$ .50	Perú-Chile: II–IV
<i>Cistanthe grandiflora</i> (Lindl.) Carolin ex Hershk	Portulacaceae	Annual	3.87 $\pm$ .24	Chile: III–VII
<i>Cistanthe longiscapa</i> (Barnéoud) Carolin ex Hershk	Portulacaceae	Annual	2.20 $\pm$ .32	Chile: III–IV
<i>Nicotiana solanifolia</i> Walp.	Nolanaceae	Shrub	.97 $\pm$ .03	Chile: II–III
<i>Nolana divaricata</i> (Lindl.) Johnst	Nolanaceae	Shrub	.93 $\pm$ .15	Chile: II–III
<i>Nolana rupicola</i> Gaudich.	Nolanaceae	Shrub	3.98 $\pm$ .01	Chile: II–IV
<i>Oenothera coquimbensis</i> Gay	Onagraceae	Annual	2.23 $\pm$ .15	Chile: II–V
<i>Quinchamalium chilense</i> Mol.	Santalaceae	Perennial herb	2.24 $\pm$ .40	Chile: II–XI
<i>Rhodophiala bagnoldii</i> (Herb.) Traub	Amaryllidaceae	Perennial herb	5.63 $\pm$ .30	Chile: II–III
<i>Schizanthus candidus</i> Lindl.	Solanaceae	Annual	2.28 $\pm$ .12	Chile: III–IV
<i>Solanum heterantherum</i> Witasek & Reiche	Solanaceae	Shrub	1.68 $\pm$ .06	Chile: III–IV
<i>Solanum remyanum</i> Phil.	Solanaceae	Shrub	1.58 $\pm$ .12	Chile: II–III
<i>Zephyra elegans</i> D. Don	Tecophilaeaceae	Perennial herb	1.08 $\pm$ .04	Chile: II–III

Note. Life form (annual, perennial herb, and shrub), flower size (cm), and distribution range are shown. Distribution data show the Chilean political region(s) where the species is currently found. Note that the majority of species are endemic to the Atacama Desert, which extends from region I to region IV.

ment 4 wk after anthesis, and data across the 40 individuals were pooled. We pooled data because only a few flowers per plant were pollinated. We acknowledge that reallocation of resources from N flowers to H flowers on the same plant could lead us to overestimate PL, as only a fraction of a plant's flowers were pollinated (Knight et al. 2006). We found, however, lower levels of PL than expected in desert flora (see "Results"). Moreover, recently a meta-analysis by García-Camacho and Totland (2009) did not find differences in PL between experiments conducted on only a fraction of flowers versus all the flowers of a plant.

The following analyses were performed for each species separately. First, to test whether fruit production of desert species is pollen limited, the fruit set of treatments N and H was compared. Because both treatments were performed on the same individuals, a McNemar test (a nonparametric method to compare proportions in paired studies; McNemar 1947) was used to assess statistical significances. A PL index was calculated using the formula  $PL = 1 - N/H$  (Larson and Barrett 2000). Second, to assess whether plants have the capacity for autonomous selfing, the fruit set of autonomous-selfing (AS) flowers and that of the H flowers were compared. Because AS and H treatments were conducted in different individuals, a  $\chi^2$  test was used to compare fruit sets. An autofertility index (AFI) was calculated using the formula  $AFI = AS/H$  (Schoen and Lloyd 1992).

The following analyses were performed only for those species capable of autonomous selfing (AFI values  $>0.5$ ). First, to assess whether sufficient pollen is deposited by pollinators for full seed set in natural populations, the fruit set of E flowers was compared with the fruit set of H flowers, using a McNemar test. Because H and E flowers are under the same resource constraints, differences between treatments are expected to be the result of pollinator failure (PF; Kalisz and Vogler 2003; Kalisz et al. 2004); therefore, a PF rate was estimated as  $PF = 1 - E/H$ . Second, to test whether autonomous selfing increased seed set over vector-mediated

pollination, the fruit set of N and E flowers was compared, using a McNemar test. Because N and E flowers are under the same resource constraints and pollinator environment, differences between treatments are expected to be the result of autonomous selfing (Schoen and Lloyd 1992; Kalisz and Vogler 2003). The relative contribution of reproductive assurance (RAS) to fruit set was calculated as  $RAS = 1 - E/N$  (Schoen and Lloyd 1992). Modified Bonferroni corrections were applied for multiple comparisons (Jaccard and Wan 1996).

#### Comparative Analyses

Comparative analyses by phylogenetically independent contrasts (Felsenstein 1985; Purvis and Rambaut 1995) were performed to examine the relationships between autofertility and life form, between PL and autofertility, and between PL and flower size. A phylogenetic tree of the species in the data set was assembled using the phylogeny of angiosperms at the subfamily level, from the Angiosperm Phylogeny Group (Stevens 2001–). Except Solanaceae, all families in the data set are represented by one or two species, and hence, family information was sufficient to posit the majority of studied species. Solanaceous genera were positioned according to Olmstead and Palmer (1992). Branch lengths were set to unity. Phylogenetic independent contrasts for AFI, PL, and flower size were generated separately, using the PDAP module (Midford et al. 2003) of Mesquite (Maddison and Maddison 2007). AFI and PL were transformed by the arcseno function. No correlation between the absolute values of the contrasts and their standard deviations were found. To test whether AFI differed between annuals and perennial species, the procedure designed for discrete predictor variables of Purvis and Rambaut (1995) was used. AFI contrasts were analyzed using a *t*-test on the mean of the contrasts. A mean significantly different from 0 indicated that AFI differed between annuals and perennials. To test whether PL decreased with AFI and to test whether PL increased with flower size,

a linear regression forced through the origin was fitted. Analysis of PL versus flower size was performed with and without autonomous-selfing species, predicting that a correlation between flower size and PL would be more pronounced in non-autonomous-selfing species.

## Results

### Pollen Limitation

Plants from the coastal Atacama Desert showed a mean PL of 0.28 (0.07 SE) ranging from  $-0.04$  to  $0.74$  (table 2). The H treatment produced a significant increase in fruit set with respect to the N treatment in only six (*Alstroemeria philippi*, *Adesmia argyrophylla*, *Balsipia peduncularis*, *Nolana divaricata*, *Nolana rupicola*, *Solanum remyanum*) out of the 16 species examined, representing 37% of the total. PL indexes and *P* values of statistical differences between N and H treatments for each species are shown in table 2.

### Autofertility and Life Form

The 16 species showed a full range of autofertility levels (table 2), ranging from complete autonomous selfing ( $AFI \geq 1$ : *Oenothera coquimbensis* and *Schizanthus candidus*) to complete dependence on pollinators for seed set ( $AFI = 0$ : *Solanum heterantherum*, *B. peduncularis*, and *A. philippi*). This variation was closely related to life forms ( $n = 4$ , mean contrast =  $0.85$ ,  $P = 0.03$ , one tail). In all annual species, AFI values exceeded  $0.85$ , and the fruit set from hand-pollinated flowers was not significantly higher than the fruit set from bagged flowers ( $\chi^2$  tests; table 2). In contrast,

bagging significantly reduced the fruit set of all perennial species ( $\chi^2$  tests; table 2). Three perennial herbs (*Quinchamalium chilense*, *Rhodophiala bagnoldii*, and *Zephyra elegans*), however, showed important levels of autofertility ( $AFI \geq 0.47$ ). The levels of AFI in the other two perennial species and in shrubs did not exceed  $0.25$  (table 2). Overall, seven desert species (48%) out of 16 presented substantial levels of autofertility.

### Pollinator Failure and Reproductive Assurance

When the capacity for autonomous selfing was removed by emasculating, hand pollination produced a significant increase in fruit set in five of the seven species that showed substantial levels of autofertility (McNemar tests; table 3). These five species (*S. candidus*, *O. coquimbensis*, *Cistanthe longiscapa*, *Cistanthe grandiflora*, and *Q. chilense*) are therefore subjected to some level of pollinator failure ( $PF > 0.35$ ). In four of them, the fruit set of open-pollinated N flowers was significantly higher than the fruit set of open-pollinated E flowers (McNemar tests; table 3), indicating that autonomous selfing increased fruit production over those flowers dependent exclusively on vector-mediated pollination. The contribution of autonomous selfing ranged from  $0.08$  in *R. bagnoldii* (the species subjected to the lowest levels of PF) to  $0.89$  in *O. coquimbensis* (the species subjected to the highest levels of PF).

### Pollen Limitation, Autofertility, and Flower Size

Independent-contrast analyses revealed that PL is negatively correlated with AFI ( $n = 15$ ,  $r = -0.68$ ,  $P < 0.01$ , one

**Table 2**  
Pollen Limitation (PL) and Autofertility (AFI) in 16 Species of the Coastal Atacama Desert

	Fruit set (%)			AFI = AS/H	PL = 1 - N/H	$\chi^2$	McNemar
	AS	H	N			AS vs. H ( <i>P</i> value)	N vs. H ( <i>P</i> value)
Annuals:							
<i>Cistanthe grandiflora</i>	86	100	95	.86	.05	.03	.17
<i>Cistanthe longiscapa</i>	81	92	96	.88	-.04	.21	.43
<i>Oenothera coquimbensis</i>	100	100	100	1.00	.00	1	1
<i>Schizanthus candidus</i>	100	95	95	1.05	.00	.21	1
Perennial herbs:							
<i>Alstroemeria philippi</i>	0	69	28	.00	.59	<.001*	<.001*
<i>Alstroemeria werdermannii</i>	11	86	70	.13	.19	<.001*	.11
<i>Quinchamalium chilense</i>	54	94	74	.57	.21	<.001*	.03
<i>Rhodophiala bagnoldii</i>	60	92	79	.65	.14	.004*	.20
<i>Zephyra elegans</i>	33	70	62	.47	.11	.004*	.55
Shrubs:							
<i>Adesmia argyrophylla</i>	8	90	35	.09	.61	<.001*	<.001*
<i>Balsipia peduncularis</i>	0	86	50	.00	.42	<.001*	.001*
<i>Nicotiana solanifolia</i>	15	85	83	.18	.02	<.001*	.85
<i>Nolana divaricata</i>	16	64	21	.25	.67	<.001*	<.001*
<i>Nolana rupicola</i>	10	96	57	.10	.41	<.001*	<.001*
<i>Solanum heterantherum</i>	0	74	50	.00	.32	<.001*	.08
<i>Solanum remyanum</i>	12	80	21	.15	.74	<.001*	<.001*

Note. Fruit set of autonomous pollinated flowers (AS), supplemental hand-cross-pollinated flowers (H), and naturally pollinated flowers (N) are shown. *P* values of statistical differences in fruit set between AS and H treatments and between N and H treatments tested using  $\chi^2$  and McNemar tests, respectively, are also shown.

\* Significant differences between treatments after modified Bonferroni adjustments.

Table 3

**Pollinator Failure (PF) and the Relative Contribution of Autonomous Selfing (RAS) to Fruit Production in the Seven Species from the Atacama Desert That Showed Substantial Levels of Autofertility (AFI  $\geq$  0.47)**

	E fruit set (%)	PF = 1 - E/H	RAS = 1 - E/N	H vs. E ( <i>P</i> value)	N vs. E ( <i>P</i> value)
<i>Cistanthe grandiflora</i>	65	.35	.32	<.001*	.002*
<i>Cistanthe longiscapa</i>	42	.54	.56	<.001*	<.001*
<i>Oenothera coquimbensis</i>	11	.89	.89	<.001*	<.001*
<i>Shizanthus candidus</i>	57	.40	.41	<.001*	<.001*
<i>Quinchamalium chilense</i>	47	.50	.36	<.001*	.01
<i>Rhodophiala bagnoldii</i>	72	.22	.08	.04	.55
<i>Zephyra elegans</i>	48	.31	.22	.08	.30

Note. Fruit set of open-pollinated emasculated flowers (E) is shown. H = supplemental hand-cross-pollinated flowers; N = naturally pollinated flowers.

\* Statistical differences in fruit set between E and N treatments after Bonferroni adjustments.

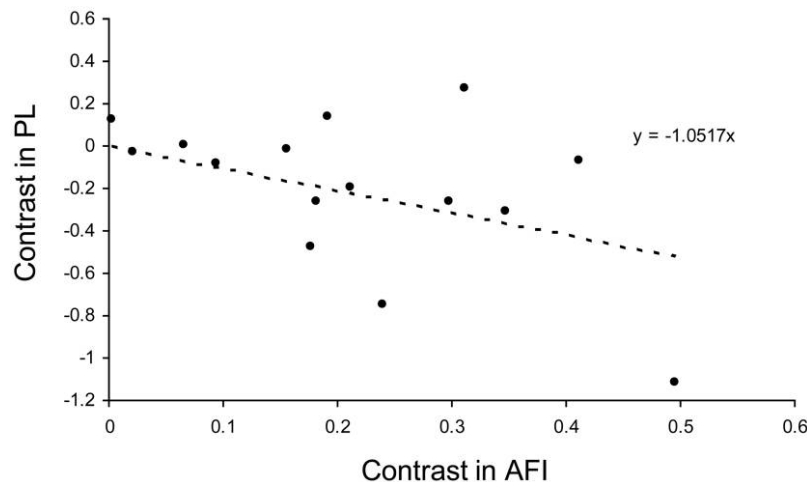
tail; fig. 1). The mean PL of the species strongly dependent on pollinators for seed set was six times greater than that for plants capable of autonomous selfing (mean PL = 0.44 for species with AFI < 0.25 vs. mean PL = 0.07 for species with AFI > 0.40). Independent-contrast analyses between PL and flower size showed no association between these variables both when analyzing all species ( $n = 15$ ,  $r = -0.05$ ,  $P = 0.83$ ) and when considering only non-autonomous-selfing species ( $n = 8$ ,  $r = -0.09$ ,  $P = 0.41$ ).

### Discussion

Given the high interannual variation in rainfall and plant cover and phenology in the coastal Atacama Desert and in accordance with the bet-hedging hypothesis (Burd 1995), we expected that the reproduction of desert plants would be strongly limited by pollen. Contrary to our expectations, the percentage of species (37%) subjected to significant levels of PL and the mean PL (0.28) observed in this desert community were lower than those reported for angiosperms in extensive data sets across several biomes (Burd 1994; Larson

and Barrett 2000; Knight et al. 2005). For example, Knight et al. (2005) reported that fruit set was significantly limited by pollen in 63% of 483 taxa, while Larson and Barrett (2000) reported a mean PL of 0.40 for 224 species.

Low levels of PL can be attributed to the high incidence of autofertility in desert plants, which was strongly correlated with life form. Several studies have shown that self-fertile taxa are common in habitats where pollinators appear to be scarce, including, for example, habitats with severe weather conditions (Hagerup 1951; Garnock-Jones 1976; Lloyd 1979), oceanic islands (Baker 1955), and peripheral populations (Wyatt 1986). We found that PL was negatively correlated with autofertility and that PL of non-autonomous-selfing species was six times greater than that of plants capable of autonomous selfing. This difference was higher than that reported by Larson and Barrett (2000), who found that PL in non-autonomous-selfing species was twice that of autonomous-selfing species in several biomes. In the majority of autonomous-selfing desert species analyzed here, supplementary hand pollination increased significantly the fruit set over vector-mediated pollination when their autonomous



**Fig. 1** Relationship between independent contrast in autofertility (AFI) and pollen limitation (PL). A linear regression forced through the origin was fitted.

selfing capacity was removed by emasculation, indicating that no sufficient pollen is deposited by pollinators to full fruit set and that species are subjected to some level of pollinator failure. These results suggest that the low levels of PL in autonomous-selfing desert species resulted from the reproductive assurance provided by selfing and not from a reliable pollinator service. Accordingly, low visitation rates had been observed in the region (Pérez et al. 2006; R. Medel, unpublished data). Nevertheless, more pollinator data are necessary to confirm this hypothesis. We found that the contribution of autonomous selfing was greater when plants were subjected to higher levels of pollinator failure. These results agree with those of other studies showing that self-fertilization provided reproductive assurance when vector-mediated pollination limited seed production (Piper et al. 1986; Herlihy and Eckert 2002; Kalisz and Vogler 2003; Pérez et al. 2009). In other cases, vector-mediated pollination did not limit seed production, and self-fertilization provided no reproductive assurance (Klips and Snow 1997; Eckert and Schaefer 1998).

As pointed out above, autofertility was strongly associated with life form. Whereas all annual species presented autonomous selfing, the majority of perennial species were dependent on pollinators. The association between life form and autonomous selfing has been routinely demonstrated for other communities, including montane sclerophyllous forest (Arroyo and Uslar 1993) and alpine shrublands (Arroyo and Squeo 1990). In general terms, annual species are more likely to self-fertilize than perennial species (Morgan et al. 1997) because the reproductive assurance provided by selfing would have less significance in long-lived perennials. In perennial plants, a failure to achieve successful reproduction in 1 yr would not compromise the long-term survival of the species. Furthermore, perennials would suffer more inbreeding depression and seed discount than annuals (Morgan et al. 1997).

Considering non-autonomous-selfing desert species only, the mean PL (0.44) was similar to that reported by Larson and Barrett (2000) for non-autonomous-selfing species in a data set across several biomes (mean = 0.37,  $n = 97$ ). This result shows that, as in the majority of angiosperms, plant populations in the Atacama Desert are pollen limited. Nevertheless, the magnitude and incidence of PL were not as high as expected, given the highly unpredictable and severe conditions of the hyperarid Atacama Desert. These results do not support the bet-hedging hypothesis but do not rule it out, either. Although the bet-hedging hypothesis predicts that PL should be particularly strong in variable pollinator environments over many years, low levels of PL would occur during years of abundant pollination (Burd 1995). Long-term studies are necessary to examine the interannual variation of PL in the Atacama Desert and thus determine whether the moderate levels of PL that we found during 2008 in non-autonomous-selfing species is recurrent or, conversely, whether the extent of PL covaries with rainfall. Rainfall during 2008 was sufficient to support the growth of annuals and perennial herbs and also the flowering of shrubs, which remain dormant during dry years, although annual rainfall was about one-half of that recorded during years of above-average rainfall associated with known ENSO events (e.g.,

1992, 1997, 2002). Levels of PL even lower than we reported for 2008 would occur during high-rainfall years (ENSO events), whereas drier years would trigger limited or no germination and growth of annual plants.

Although it has often been claimed that PL should be higher under ecological conditions that limit biotic pollination or increase their unpredictability (Burd 1995; see also Larson and Barrett 2000; Wilcock and Neiland 2002), the few studies that compare PL between species occurring in contrasting environments failed to find strong differences between habitats. In a comparative study, Larson and Barrett (2000), using phylogenetic independent contrast, did not find differences between plants from open and forested habitats or between tropical and temperate sites. Similarly, García-Camacho and Totland (2009) found no differences in PL between lowland and alpine species. The lack of differences between habitats was attributed to several factors that may compensate the low availability of pollinators at higher altitudes, including extended flower longevity of alpine flowers (Arroyo et al. 1985) and a major efficiency of alpine pollinators (Arroyo et al. 1985) compared to those at lower altitudes.

We did not detect an association between PL and flower size, either for all the species studied or for non-autonomous-selfing species only. Our results agree with those of Knight et al. (2005), who failed to detect an association between PL and flower size in a comparative study across many species. We expected that desert species with larger flowers could be less pollen limited than species with smaller flowers, assuming that large flowers enhance the ability of plants to attract the few pollinators available. Pollinator attraction, however, could also be affected by traits that were not considered in our study, such as flower number, nectar production, flower color, or plant density. Even though, intuitively, PL should increase for plants that attract more pollinators, this relationship is predicted to change in variable environments (Burd 2008). Haig and Westoby (1988) proposed that plants living in constant environments should evolve floral traits that increase pollinator attraction, reducing PL until an equilibrium is reached, at which point constraints other than pollen availability would limit seed set. Burd (2008) incorporated stochasticity in ovule fertilization and resource availability to Haig and Westoby's (1988) model and showed that the degree of PL can be greater when plants attract more pollinators.

Overall, plant species inhabiting an extremely variable environment such as the Atacama Desert do not show the expected high levels of PL. Long-term studies are necessary to examine how interannual variation in rainfall, plant cover, and pollinators could affect PL and its relation with traits involved in reducing these effects.

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### Literature Cited

- Arroyo MTK, JJ Armesto, RB Primack 1985 Community studies in population ecology in the high temperate Andes of central Chile. II. Effect of temperature on visitation rates and pollination possibilities. *Plant Syst Evol* 149:187–203.
- Arroyo MTK, FA Squeo 1990 Relationship between plant breeding systems and pollination. Pages 205–227 in S Kawano, ed. *Biological approaches and evolutionary trends in plants*. Academic Press, New York.
- Arroyo MTK, P Uslar 1993 Breeding systems in a temperate Mediterranean-type climate montane sclerophyllous forest in central Chile. *Bot J Linn Soc* 111:83–102.
- Ashman TL, TM Knight, JA Steets, P Amarasekare, M Burd, DR Campbell, MR Dudash, et al 2004 Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85:2408–2421.
- Baker HG 1955 Self-compatibility and establishment after long-distance dispersal. *Evolution* 9:347–349.
- Billings WD, HA Mooney 1968 The ecology of arctic and alpine plants. *Biol Rev* 43:481–529.
- Burd M 1994 Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot Rev* 60:83–139.
- 1995 Ovule packaging in stochastic pollination and fertilization environments. *Evolution* 49:100–109.
- 2008 The Haig-Westoby model revisited. *Am Nat* 171:400–404.
- Burd M, TL Ashman, DR Campbell, MR Dudash, MO Johnston, TM Knight, S Mazer, R Mitchell, J Steets, JC Vamosi 2009 Ovule number per flower in a world of unpredictable pollination. *Am J Bot* 96:1159–1167.
- Di Castri F, ER Hajek 1976 Bioclimatología de Chile. Editorial Pontificia Universidad Católica de Chile, Santiago de Chile.
- Eckert CG, A Schaefer 1998 Does self-pollination provide reproductive assurance in wild columbine, *Aquilegia canadensis* (Ranunculaceae)? *Am J Bot* 85:919–924.
- Fabbro T, C Körner 2004 Altitudinal differences in flower traits and reproductive allocation. *Flora* 199:70–81.
- Felsenstein J 1985 Phylogenies and the comparative method. *Am Nat* 125:1–15.
- García-Camacho R, Ø Totland 2009 Pollen limitation in the alpine: a meta-analysis. *Arct Antarct Alp Res* 41:103–111.
- Garnock-Jones PJ 1976 Breeding systems and pollination in New Zealand: *Parahebe* (Scrophulariaceae). *N Z J Bot* 14:291–298.
- Goodwillie C, S Kalisz, CG Eckert 2005 The evolutionary enigma of mixed mating system in plants: occurrence, theoretical explanations, and empirical evidence. *Annu Rev Ecol Syst* 36:47–79.
- Hagerup O 1951 Pollination in the Faroes: in spite of rain and poverty in insects. *Dan Biol Medd* 15:1–48.
- Haig D, M Westoby 1988 On limits of seed production. *Am Nat* 131:757–759.
- Herlihy CR, CG Eckert 2002 Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416:320–323.
- Holmgren M, P Stapp, CR Dickman, C Gracia, S Graham, JR Gutiérrez, C Hice, et al 2006 A synthesis of ENSO effects on drylands in Australia, North America and South America. *Adgeo* 6:69–72.
- Jaccard J, CK Wan 1996 LISREL approaches to interaction effects in multiple regression. Sage, Thousand Oaks, CA.
- Kalisz S, DW Vogler 2003 Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84:2928–2942.
- Kalisz S, DW Vogler, KM Habkey 2004 Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430:884–887.
- Klips RA, AA Snow 1997 Delayed autonomous self-pollination in *Hibiscus laevis* (Malvaceae). *Am J Bot* 84:48–53.
- Knight TM, JA Steets, TL Ashman 2006 A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *Am J Bot* 93:271–277.
- Knight TM, JA Steets, JC Vamosi, SJ Mazer, M Burd, DR Campbell, MR Dudash, MO Johnson, RJ Mitchell, TL Ashman 2005 Pollen limitation of plant reproduction: pattern and process. *Annu Rev Ecol Syst* 36:467–497.
- Larson BM, SCH Barrett 2000 A comparative analysis of pollen limitation in flowering plants. *Biol J Linn Soc* 69:503–520.
- Lloyd DG 1979 Some reproductive factors affecting the selection of self-fertilization in plants. *Am Nat* 113:67–79.
- 1992 Self- and cross-fertilization in plants. II. The selection of self-fertilization. *Int J Plant Sci* 153:370–380.
- Maddison WP, DR Maddison 2007 Mesquite: a modular system for evolutionary analysis, version 2.0. <http://www.mesquiteproject.org>.
- McNemar Q 1947 Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika* 12:153–157.
- Midford PE, T Garland, WP Maddison 2003 PDAP: PDTREE package for Mesquite, version 1.12. [http://mesquiteproject.org/ pdap\\_mesquite/](http://mesquiteproject.org/ pdap_mesquite/).
- Morgan MT, DJ Schoen, TM Bataillon 1997 The evolution of self-fertilization in perennials. *Am Nat* 150:618–638.
- Morgan MT, WG Wilson 2005 Self fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* 59:1143–1148.
- Olmstead RG, JD Palmer 1992 A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. *Ann Mo Bot Gard* 79:346–360.
- Pérez F, MTK Arroyo, JJ Armesto 2009 Evolution of autonomous selfing accompanies increased specialization in the pollination system of *Schizanthus* (Solanaceae). *Am J Bot* 96:1168–1176.
- Pérez F, MTK Arroyo, R Medel, M Herskovitz 2006 Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). *Am J Bot* 93:1029–1038.
- Petit RJ, A Hampe 2006 Some evolutionary consequences of being a tree. *Annu Rev Ecol Syst* 37:187–214.
- Piper JG, B Charlesworth, D Charlesworth 1986 Breeding system evolution in *Primula vulgaris* and the role of reproductive assurance. *Heredity* 56:207–217.
- Purvis A, A Rambaut 1995 Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comput Appl Biosci* 11:247–251.

- Schoen DJ, DG Lloyd 1992 Self-fertilization and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *Int J Plant Sci* 153:381–393.
- Stebbins GL 1974 Flowering plants: evolution above the species level. Harvard University Press, Cambridge, MA.
- Stevens PF 2001– Angiosperm phylogeny Web site, version 9, June 2008. <http://www.mobot.org/MOBOT/research/APweb/>.
- Stone JL, EG Jenkins 2007 Pollinator abundance and pollen limitation of a solanaceous shrub at premontane and lower montane sites. *Biotropica* 40:55–61.
- Toro H, E Chiappa, R Covarrubias 1996 Diversidad de Apoidea y su asociación a la vegetación nativa del norte de Chile. II. Región. *Rev Chil Entomol* 23:65–81.
- Vidiella PE, JJ Armesto 1989 Emergence of ephemeral plant species from the north-central Chilean desert in response to experimental irrigation. *Rev Chil Hist Nat* 62:99–107.
- Wilcock C, R Neiland 2002 Pollination failure in plants: why it happens and when it matters. *Trends Plant Sci* 7:270–277.
- Wyatt R 1986 Ecology and evolution of self-pollination in *Arenaria uniflora* (Caryophyllaceae). *J Ecol* 74:403–418.