

Temperature-driven flower longevity in a high-alpine species of *Oxalis* influences reproductive assurance

Mary T. K. Arroyo^{1,2}, Leah S. Dudley³, Gus Jespersen⁴, Diego A. Pacheco^{1,2} and Lohengrin A. Cavieres^{1,5}

¹Institute of Ecology and Biodiversity (IEB), Santiago, Chile; ²Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile; ³Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA; ⁴School of Environmental and Forest Sciences, University of Washington, WA 98195, USA;

⁵Departamento de Botánica, Universidad de Concepción, Concepción, Chile

Author for correspondence:

Mary T. K. Arroyo

Tel: +1 56 2 2715464

Email: southern@uchile.cl

Received: 12 May 2013

Accepted: 3 July 2013

New Phytologist (2013) **200**: 1260–1268

doi: 10.1111/nph.12443

Key words: adaptive plasticity, Andes, flower longevity, global warming, high alpine, *Oxalis*, self-incompatibility, thermal conditions.

Summary

- How high-alpine plants confront stochastic conditions for animal pollination is a critical question. We investigated the effect of temperature on potential flower longevity (FL) measured in pollinator-excluded flowers and actual FL measured in pollinated flowers in self-incompatible *Oxalis compacta* and evaluated if plastically prolonged potential FL can ameliorate slow pollination under cool conditions.
- Pollinator-excluded and hand-pollinated flowers were experimentally warmed with open-top chambers (OTCs) on a site at 3470 m above sea level (asl). Flower-specific temperatures, and pollinator-excluded and open-pollination flower life-spans were measured at six alpine sites between 3100 and 3470 m asl. Fruit set was analyzed in relation to inferred pollination time.
- Warming reduced potential FL. Variable thermal conditions across the alpine landscape predicted potential and actual FL; flower senescence was pollination-regulated. Actual FL and potential FL were coupled. Prolonged potential FL generally increased fruit set under cooler conditions.
- Plastic responses permit virgin flowers of *O. compacta* to remain open longer under cooler temperatures, thereby ameliorating slow pollination, and to close earlier when pollination tends to be faster under warmer conditions. Plastic potential FL provides adaptive advantages in the cold, thermally variable alpine habitat, and has important implications for reproductive success in alpine plants in a warming world.

Introduction

The globally distributed alpine life-zone is characterized by cold air temperatures, variable weather, and high thermal variation (Rozzi *et al.*, 1997; Torres-Díaz *et al.*, 2007; Graae *et al.*, 2011; Scherrer & Körner, 2011). These conditions together produce impoverished, temporally unpredictable, and spatially variable conditions for animal pollination across the rugged alpine landscape (Arroyo *et al.*, 1985; Inouye & Pyke, 1988; Arroyo & Squeo, 1990; McCall & Primack, 1992; Totland, 1993, 1994; Bergman *et al.*, 1996; Rozzi *et al.*, 1997; Utelli & Roy, 2000; Lundemo & Totland, 2007; Torres-Díaz *et al.*, 2007; Pérez *et al.*, 2009). A central question in alpine ecology concerns how animal-pollinated species deal with low and unpredictable pollinator visitation. This question is not only of general theoretical interest in relation to its implications for plant breeding system evolution, but also has ramifications for alpine plant species persistence in a warming world.

Autonomous self-pollination (Arroyo *et al.*, 2006; Zhang & Li, 2008), apomixis (Hörandl *et al.*, 2011), late-acting selfing (Pérez *et al.*, 2009) and abandonment of animal pollination for

wind pollination (Arroyo & Squeo, 1987; Sobrevila, 1989; Totland & Sottocornola, 2001; Duan *et al.*, 2009; Gulías & Traveset, 2012) provide reproductive assurance in many alpine species. However, a significant number of highly genetically self-incompatible species in the alpine zone are dependent on animal pollination (Arroyo & Squeo, 1990; García-Camacho & Totland, 2009).

One flower trait that is likely to be important for successful sexual reproduction in highly self-incompatible, animal-pollinated alpine species in a stochastic pollination environment is flower longevity (FL; Arroyo *et al.*, 2006). Kerner von Marilaun (1895) held that plants in general with long-lived flowers have adapted to unpredictable weather that is not often suitable for pollinator visitation. The longer virgin flowers are able to remain open and receptive to pollen, the greater the chances of successful pollen importation and exportation (Primack, 1985; Ashman & Schoen, 1996; Rathcke, 2003). Two expressions of FL are recognized. Potential FL represents the maximum time a virgin flower is able to stay open in the absence of pollination (Steinacher & Wagner, 2010) and should be distinguished from actual FL, which refers to the time open- or hand-pollinated flowers remain

open. Because pollination in many species provokes flower senescence (van Doorn, 1997; O'Neill, 1997), tendencies in actual FL do not necessarily reflect tendencies in potential FL.

Prolonged potential FL in the alpine habitat could arise in various ways. The wide range of flower life-spans found in flowering plants is thought to be shaped by natural selection to maximize pollinator visitation while minimizing flower maintenance costs (Ashman & Schoen, 1996). The longer potential flower life-spans of alpine populations of *Aconitum lycoctonum* (Ranunculaceae) in the European Alps (Utelli & Roy, 2000) and extremely long potential capitulum life-spans of high-alpine *Chaetanthera renifolia* (Asteraceae) in the central Chilean Andes (Torres-Díaz *et al.*, 2011) are thought to reflect selection on FL mediated by pollinators. As an alternative to selection, prolonged potential flower life-spans in the alpine zone could result from slower flower development under cooler ambient temperatures and thus reflect a noninheritable plastic response (Arroyo *et al.*, 1981; Steinacher & Wagner, 2010). Controlled laboratory experiments show that virgin FL is more prolonged under cooler temperatures (Hartley *et al.*, 1995; Yasaka *et al.*, 1998; Lopez & Runkle, 2004; Vesprini & Pacini, 2005). Prolonged pollinator-excluded flower life-spans at higher, cooler elevations along extensive altitudinal gradients (Utelli & Roy, 2000; Blionis & Vokou, 2001; Giblin, 2005) might be attributable, at least partially, to slower flower development. Interestingly, Duan *et al.* (2007) found a nonsignificant difference in potential FL in an alpine *Gentiana straminea* for sites at two different altitudes on the Qinghai-Tibetan Plateau but with very similar temperatures.

If the flower life-spans of virgin flowers can be plastically prolonged under cool temperatures, and cool temperatures are associated with stochastic pollination conditions, temperature-driven potential FL could provide a flexible and highly efficient means of assuring reproduction in pollinator-dependent, self-incompatible species growing in the alpine zone. Moreover, if temperature drives both potential FL and pollinator visitation, potential FL is expected to be coupled with actual FL as measured in pollinated flowers.

Here, we examined the effect of temperature on FL and the consequences of plastic FL for reproductive success in a strongly outcrossing, insect-pollinated high-alpine species. *Oxalis compacta* ssp. *compacta* occurs on a wide variety of substrates with different thermal properties and inhabits sparsely vegetated slopes of different slope/aspect in the central Chilean Andes (33°S) between 3100 and 3650 m above sea level (asl). For *O. compacta* ssp. *compacta*, we asked: does potential FL show plastic responses to ambient temperature? Is actual FL pollination-regulated? Does variation in ambient temperature explain natural variation in potential and actual FL? Can prolonged potential FL ameliorate slow pollinator visitation under cooler temperatures? Is pollination concentrated early in the flower life-span under warm conditions? To answer these questions, we warmed naturally occurring plants in the field with open-top chambers (OTCs) commonly used in climate warming research. We then submitted warmed and ambient-temperature flowers to pollinator exclusion and hand pollination. We measured potential FL in pollinator-excluded flowers and actual FL in open-pollinated flowers at six

alpine sites and recorded microsite temperature adjacent to each plant to quantify the thermal environment around individual flowers. We investigated the effect of virgin flower life-spans of different lengths on pollination schedules by constructing fruit set accumulation curves as a function of the inferred time of pollination in flowers.

Global warming is forcing plant species upwards in the alpine zone (Gottfried *et al.*, 2012) and provoking earlier flowering (Inouye, 2008; Anderson *et al.*, 2012). High plasticity in reproductive traits should be advantageous under global warming because ecologically relevant variation can arise rapidly in response to new environmental cues (Nicotra *et al.*, 2010; Levin, 2012). By employing OTCs used extensively in climate change research as a supplementary heat source and searching for links between natural thermal variation and FL, in addition to investigating a central question in alpine ecology, we obtained valuable insights into how FL and fruit and seed set are likely to be affected by the warming of high-alpine habitats as a result of global climate change.

Materials and Methods

Study species and area

Oxalis compacta Gill. ex Hook. et Arn. ssp. *compacta* (Oxalidaceae; hereafter *O. compacta*; Fig. 1) is a perennial herb that occurs on bare substrates in the central Chilean Andes. On our study sites in the Andes it is entirely restricted to the upper alpine zone between 3100 and 3650 m asl. *Oxalis compacta* is tristylous. Although some seed set can occur in illegitimate crosses (requiring cross-pollination) and via sporadic autonomous self-pollination, *O. compacta* is highly self-incompatible (Arroyo *et al.*, 2012). Fruit set does not differ between floral morphs (Arroyo *et al.*, 2012). Individual flowers of *O. compacta* open in early/mid-morning and close in the mid/late afternoon on each day of the flower's life-span. Anthers begin dehiscing just before, or as, the flowers begin to open for the first time. Using the peroxidase test (Kearns & Inouye, 1993), stigmas on flowers were found to be receptive throughout the flower life-span. This was



Fig. 1 *Oxalis compacta* growing in the high-alpine zone in the central Andes of Chile at 3470 m above sea level (asl) on a typically bare, rocky substrate with poor soil development.

verified in a field experiment carried out at 3100 m asl ($n=26$ flowers), in which high levels of fruit set were always obtained when hand pollination was carried out on different days of the flower life-span (M. T. K. Arroyo, unpublished). *Oxalis compacta*'s main pollinators are low-foraging, high-Andean butterflies and minute andrenid bees (Arroyo *et al.*, 1982; Domínguez, 2010). Flower visitation rates vary widely among sites (*c.* 0–0.011 visits min^{-1}), with a mean of 0.003 visits min^{-1} (Domínguez, 2010). The mean visitation rate in *O. compacta* is very similar to the community-level rate for the upper alpine zone in the same general area (Arroyo *et al.*, 1985), which is 50% lower than in the subalpine belt found at an altitude of 1000 m below the upper alpine zone.

Work was carried out in the Andes immediately to the east of the city of Santiago (33°S) at six high-alpine sites (Table 1; Supporting Information Fig. S1) that encompass the environmental variation over which *O. compacta* is abundantly found in the high-alpine zone. General information and vegetation characteristics for the study area can be found in Arroyo *et al.* (1981) and Cavieres *et al.* (2000). The mean annual temperature is 3.6°C at 3100 m asl and 1.2°C at 3500 m asl (Cavieres *et al.*, 2000). Summers are long and fairly sunny.

Flower-warming experiment

Fifty plants were haphazardly selected at site 6 at 3470 m asl in early February 2010. Twenty-five were assigned to OTCs (supplemental warming) and 25 were left as controls (ambient temperature). The OTC model employed is identical to that used by Sierra-Almeida & Cavieres (2010) and comprises hexagonal chambers made of transparent Plexiglass[®] measuring 50 cm in height and 120 cm in diameter and open at the top. Each OTC panel contained 25 holes, 1.5 cm in diameter, to prevent excessive heating on the sparsely vegetated high-alpine slopes. As a high density of these large OTCs could affect the climate around the ambient-temperature plants, only 13 OTCs were used, 12 of which contained two plants and one of which contained a single plant (the unit of replication was the individual plant; OTCs had no effect on the variables of interest). On the same day the OTCs were installed, six mature flower buds per plant were tagged in the two temperature treatments with different colored threads in

order to be able to follow each flower over its entire life-span. All plants were covered with small pollination enclosures (*c.* 15 cm high) made of transparent muslin supported by two lateral wooden skewers, mounted on a thin plastic ring forced into the ground, from the time buds were marked until the fruiting stage. Half of the tagged flower buds on a plant were randomly assigned to a hand cross-pollination treatment and the other half to a pollinator-exclusion treatment. Hand cross-pollination took place on the day of anthesis in the early/mid-morning. Flower size has been shown to affect FL (Stratton, 1989). We therefore measured flower diameter to the nearest 0.5 mm at around midday on first-day flowers. Flowers were monitored daily from the time the buds opened to final senescence to give FL in number of days. Fruits formed were harvested *c.* 3 wk later and seed per fruit in the hand-pollinated flowers determined in order to ascertain that warming did not interfere with pollination success. Manipulation of the short- and mid-styled flowers of *O. compacta* during hand pollination causes damage to the delicate corollas; thus, only long-style plants were considered in this part of the work.

To determine the increase in temperature in the OTCs, temperature was recorded at 15-min intervals alongside each ambient-temperature and warmed plant using data loggers (HOBO U23 Pro v2; Onset Computer Corp., Cape Cod, MA, USA) fitted with shades. Loggers were positioned at a horizontal distance of 15 cm from a plant in order to avoid plant shading and the sensors were carefully leveled to 15 cm above ground level (agl), which placed them *c.* 7–10 cm above plant height. We used this logger height to be consistent with that in the survey of longevity at the six alpine sites.

For each plant in the flower-warming experiment, an average for the variables of interest was calculated for the surviving treatment flowers on a plant ($n=2-3$). An analysis of covariance (ANCOVA) was used to analyze the variation in FL as affected by temperature treatment (ambient or warmed) and pollination treatment (open-pollinated or pollinator-excluded), with flower diameter as a covariate. Two pollinator-excluded flowers that formed fruits were eliminated. Variation in seed production (seeds per fruit) among treatments was evaluated using an analysis of covariance (ANCOVA) in which temperature treatment (ambient or warmed) was treated as a fixed effect and allowed to interact with the covariate, flower diameter (mm). Flower size

Table 1 Site details for *Oxalis compacta* in the central Andes of Chile

Site	Altitude (m asl)	Latitude (°S)	Longitude (°W)	Aspect	Sample size (plants)	Daily temperature (°C) (mean (range))
1	3100	33°19'50.84"	70°16'17.66"	W-WSW	48	7.6 (6.5–8.4)
2	3450	33°19'24.57"	70°15'47.40"	WNW-NNE	49	11.1 (9.8–12.5)
3	3140	33°20'40.23"	70°15'10.67"	W-WNW	29	9.6 (8.7–11.0)
4	3450	33°19'31.40"	70°15'18.30"	N-NNE	50	10.3 (9.7–11.1)
5	3110	33°20'04.40"	70°14'38.94"	S-ESE	50	7.6 (7.0–10.1)
6	3470	33°19'14.87"	70°14'20.25"	W-WSW	50	11.4 (10.7–12.1)

Flower longevity (FL) was surveyed at all six sites. Sample sizes are final numbers of plants analyzed in the site survey of FL. Daily temperature is the average 24-h temperature recorded at 15 cm above ground level (cm agl) by all data loggers at a site over the dates FL was surveyed at that site. The flower-warming experiment was conducted only at site 6 (see text for details of temperature and sample size for this experiment). See also Supporting Information Fig. S1 for a location map of sites. asl, above sea level.

was considered as a covariate, because larger flowers could be expected to have more ovules and more seeds.

Potential and actual FL

Over the austral summer of 2009–2010, at each of the six high-alpine sites (Table 1), 50 plants were haphazardly selected, of which 25 were randomly assigned to a pollination-exclusion treatment (using the enclosures described in the previous section) and 25 to an open-pollination treatment (lacking enclosures). Five mature flower buds per plant (covering plant positional variation) were tagged on the same day for all plants. At two sites where plants were smaller on average, five large buds could not always be found (site 2, range 2–5, mean 3.3 buds; site 5, range 3–5, mean 3.8 buds). Flower diameter was again measured around midday on first-day flowers. Flowers were monitored daily from the time the flowers opened until definitive closure. Again, temperature was recorded at 15-min intervals alongside each plant in the pollinator-excluded and open-pollination treatments, positioning the loggers in the same way as described in the warming experiment. This logger height, in addition to representing the temperature conditions under which flowers develop, was representative of temperature conditions under which *O. compacta*'s pollinators are active. As the experiments required daily attention and ran for an average of 6.3 d per site (with 2–3 additional days for implementation), and only 50 data loggers were available, the six sites were studied at different times over the summer period (late December to mid-March).

Following the massive 8.8 Richter scale Chilean earthquake (27 February 2010), the entire study area became inaccessible for 3 d. As a result, we were forced to estimate the dates of closure of 63 flowers at site 5 on the basis of the degree of twisting and drying of the senescent corolla. At the time of the quake, 4 d of flower monitoring had already been completed and the remaining flowers were well along the way toward closing. Insufficient memory in the data loggers over some days at site 3 reduced the sample sizes. Twenty-two pollinator-excluded flowers at the six sites that formed fruits as a result of sporadic selfing or possibly accidental pollination by very fast andrenid bees when the pollinator enclosures were lifted each day were eliminated from the analyses. Final sample sizes taking into account the last-mentioned pollinated flowers, flower elimination and some flower breakages are given in Table 1.

A mixed model ANCOVA was used to test for effects on FL across the six alpine sites. Site was a random block effect. Pollination treatment (excluded or open) was a fixed effect. Daily 24-h temperature at 15 cm agl and flower diameter were used as continuous covariates and allowed to fully interact with each other and pollination treatment. Again, plant averages for surviving flowers (range 1–5 and mean 4.2 per plant) were calculated for each variable. For temperature, daily 24-h temperatures based on the recordings at 15-min intervals for the precise days over which each monitored flower on a plant remained open were obtained from the data loggers and averaged.

In the survey of FL at the six sites, flowers observed for potential FL but not actual FL were maintained in pollinator

enclosures. In order to assess a possible effect of the pollinator enclosures on temperature and hence on the difference between the longevity of open-pollinated and pollinator-excluded flowers, loggers fitted with pollinator enclosures alternating with loggers lacking enclosures were set up at lineal distances of 70 cm on a flat, uniform sandy terrace close to site 3, for a total of 10 loggers. The temperature sensors (recording at 15-min intervals over 3 d) were positioned at 15 cm agl inside the pollination enclosures by introducing them into small holes cut in the top of the muslin pollination bags. An average of all observations per logger was calculated. A one-way ANOVA tested for the influence of pollinator enclosure on temperature.

Fruit set for the same six sets of open-pollinated flowers was reported in Arroyo *et al.* (2012). Using these data, we determined seed number per fruit for these data to detect possible differences in pollination success in flowers among sites with different temperature conditions. If prolonged FL ameliorates slow pollination, no differences in seed per fruit are expected. Seed per fruit was analyzed in a mixed model ANCOVA in which site was treated as a random blocking effect and flower diameter, daily temperature and flower diameter \times daily temperature were continuous effects. Degrees of freedom were calculated using the Satterthwaite method, the default in JMP (SAS Institute Inc., Cary, NC, USA).

Finally, fruit set accumulation curves as a function of increasing intervals in the flower life-span over which flowers were pollinated were constructed for the six alpine sites. To pinpoint time of pollination in a flower's life-span, we relied on our characterization of post-pollination flower senescence obtained in the warming experiment (see the Results section) and the fact that actual FL can reflect when pollination takes place in a flower (Ashman & Schoen, 1996; van Doorn, 1997; O'Neill, 1997; Lundemo & Totland, 2007; Castro *et al.*, 2008). We first categorized each fruiting flower according to its open-pollinated life-span (actual FL). Actual FL was then converted into an inferred pollination interval by subtracting the number of days a flower remained open after pollination. Accumulated fruits that were inferred to have arisen from pollination occurring by a given time were expressed as a percentage of total open flowers at each site. The underlying assumption is that time of flower senescence adjusted for post-pollination flower closure lag represents the minimum time necessary for the accumulation of sufficient pollen on the stigma for ovule fertilization and for eliciting senescence.

Results

Flower-warming experiment

Mean 24-h temperatures at 15 cm agl outside and inside the OTCs were 11.3 and 14.1°C, respectively; thus the mean daily temperature increase inside the OTCs was 2.8°C. Temperature differences between the warmest and coolest days over the experimental period were similar for ambient temperature (3.6°C) and inside the OTCs (3.9°C). One of the 25 OTC plants died early in the experiment, but this was attributable to a handling problem.

Flower longevity of 1–5 d were recorded. Both the temperature and pollination treatment significantly affected FL, independently of each other (Table 2). Warming and pollination each significantly reduced flower life-span (Fig. 2). For *O. compacta*, these results reveal potential FL to be shorter when temperatures are warmer and vice versa, and flower senescence to be pollination-regulated. However, as seen in Fig. 2, flowers did not necessarily close on the day of pollination. In the ambient-temperature treatment, 23% closed on the day of pollination, 68% on the day after pollination and 9% on the second day after pollination.

Fruit was set on all hand-pollinated plants in the ambient-temperature and warmed treatments. Overall, 94.6% of ambient-temperature and 94.4% of warmed flowers formed fruits. While flower diameter (used as a proxy of flower size; range = 9.9–14.8 mm) not surprisingly had a large effect on seed production per fruit ($F_{1,45} = 4.7$; $P = 0.04$), temperature had no significant effect (all P -values > 0.1). Thus, supplemental warming did not interfere with the sequence of reproductive stages between pollen deposition and ovule fertilization.

Potential and actual FL

Temperature at 15 cm agl alongside plants was variable among (means) and within (ranges) sites (Table 1). Individual pollinator-excluded flower life-spans of 1–8 d and open-pollinated flower life-spans of 1–7 d were recorded across sites. Pollination treatment and 24-h temperature significantly affected potential and actual FL both together and independently (Table 3). Site had no effect. Higher temperatures around plants had a negative effect on both potential and actual FL (Fig. 3), indicating that potential and actual FL tended to be coupled. However, the relationship between temperature and FL was stronger for potential FL (see also Fig. 4 which gives site means for potential FL). Moreover, for similar increases in temperature, pollinator-excluded FL declined somewhat more rapidly than open-pollination FL. Overall, plants that were open-pollinated had reduced FL (least squares mean \pm 1 SE, 1.7 ± 0.2 d) compared with plants in which flowers had been pollinator-excluded (3.3 ± 0.2 d), indicating that significant amounts of pollination had taken place. Pollinator-exclusions had no significant effect on temperature ($F_{1,8} = 3.3$; $P = 0.1049$); thus, shorter actual FL in relation to potential FL is not likely to be attributable to a bagging effect on the pollinator-excluded flowers.

Table 2 Effects of temperature treatment, pollination treatment and flower diameter on flower longevity in the warming experiment on *Oxalis compacta* in the central Andes of Chile

Effect	<i>F</i>	<i>P</i>
Temperature treatment	27.8	0.0001
Pollination treatment	60.4	0.0001
Flower diameter	0.6	0.4533
Temperature \times pollination	3.4	0.0698
Temperature \times flower diameter	0.8	0.3678
Pollination \times flower diameter	1.2	0.2747
Temperature \times pollination \times flower diameter	0.2	0.6353

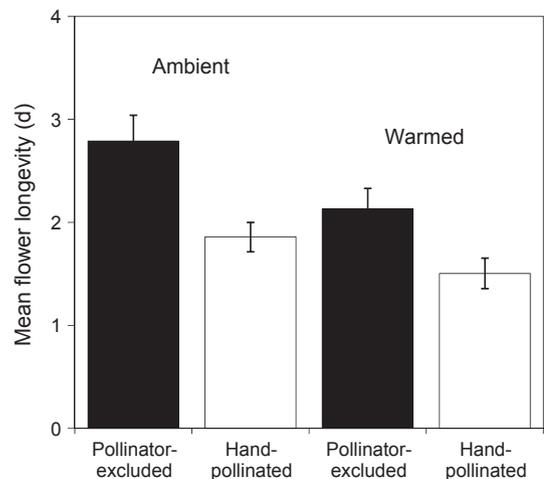


Fig. 2 Flower longevity (mean \pm 2 SE) under two temperature treatments (ambient and experimentally warmed) and two pollination treatments (hand cross-pollination and pollinator-excluded) in *Oxalis compacta*. The experiment was carried out at 3470 m above sea level (asl) in the central Andes of Chile. Supplemental warming (+ 2.8°C) was achieved with open-top chambers (OTCs).

Fig. 5 shows accumulated fruit set for flowers inferred to have been pollinated over increasing numbers of days of the flower life-span (anthesis = day 1). Because post-pollination senescence in *O. compacta* was found not always to take place on the same day as pollination, adjustment of actual FL (recorded in days) was necessary to situate inferred time of pollination in a flower. Adjustments were made by extrapolating from the distribution of flower senescence times obtained in the ambient-temperature hand-pollinated flowers. By way of example, in the pool of fruiting flowers that remained open for 5 d, 23% were considered to have been successfully pollinated by day 5, 68% by day 4, and 9% by day 3. For the three coldest sites (sites 1, 3 and 5), where potential FL was longest (cf. Fig. S2), fruits accumulated more gradually as a result of pollination taking place over several days in the flower life-span (except at site 5; Fig. 5). In contrast, at the warmest sites (sites 2, 4 and 6), where potential FL was shortest, higher amounts fruit set were concentrated and pollination completed, very early in the flower life-span. It is likely that nesting conditions for andrenid bees, which are important pollinators of

Table 3 Mixed model ANCOVA testing effects of pollination treatment, temperature at 15 cm above ground level, flower diameter and site on flower longevity in *Oxalis compacta* in the central Andes of Chile

Effect	df	<i>F</i>	<i>P</i>
Pollination treatment	264.5	317.1	0.0001
Flower diameter	266.2	0.0	0.9741
Daily temperature	94.87	13.0	0.0005
Pollination treatment \times flower diameter	263.7	3.2	0.0756
Pollination treatment \times daily temperature	264.5	6.4	0.0119
Flower diameter \times daily temperature	267.8	0.0	0.8644
Pollination treatment \times flower diameter \times daily temperature	264.1	0.8	0.3856
Site (confidence interval)		(-0.1, 0.6)	

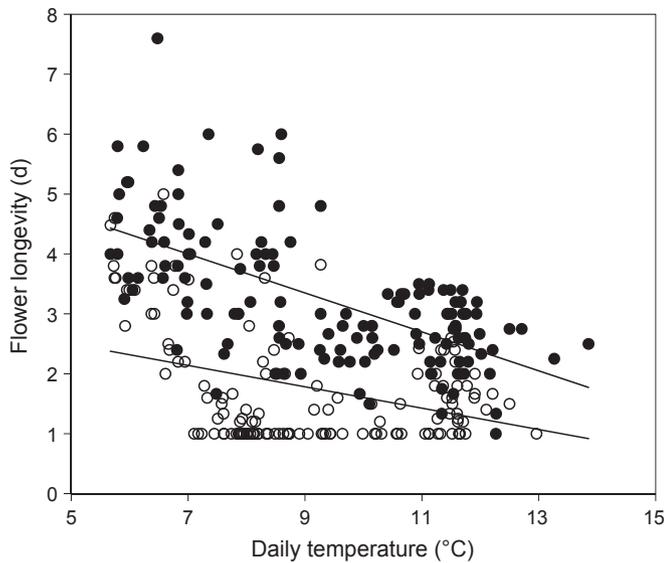


Fig. 3 Bivariate plot showing the relationship between flower longevity (FL) and 24-h temperature at 15 cm above ground level (agl) pooled across six high-alpine sites in the central Andes of Chile in *Oxalis compacta*. Closed circles, potential FL measured in pollinator-excluded flowers; open circles, actual FL measured in open-pollinated flowers. Potential FL, $y = 6.272 - 0.325x$, $R^2 = 0.39$, $F_{1,136} = 85.3$, $P < 0.0001$; actual FL, $y = 0.329 - 0.175x$, $R^2 = 0.14$, $F_{1,136} = 21.8$, $P < 0.0001$, where $y = \text{FL}$ and $x = \text{temperature}$.

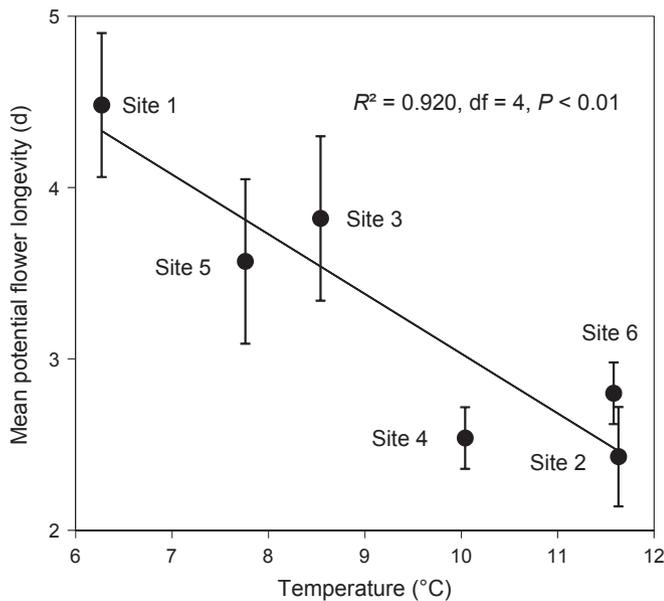


Fig. 4 Relationship between potential flower longevity (mean \pm 2 SE) and 24-h temperature at 15 cm above ground level (agl) in *Oxalis compacta* for six high-alpine sites in the central Andes of Chile. Site temperature is the average for all surveyed plants at a site over the specific days on which virgin flowers remained open ($R^2 = 0.85$, $F_{1,4} = 22.9$, $P = 0.0087$; $y = -0.35 \pm 0.07x + 6.5 \pm 0.7$).

O. compacta, were more appropriate at site 5 where, unlike the other sites, soil was well developed, leading to a higher probability of pollination earlier in the flower life-span at that particular site. Overall, the ranges for final fruit set for the three coldest

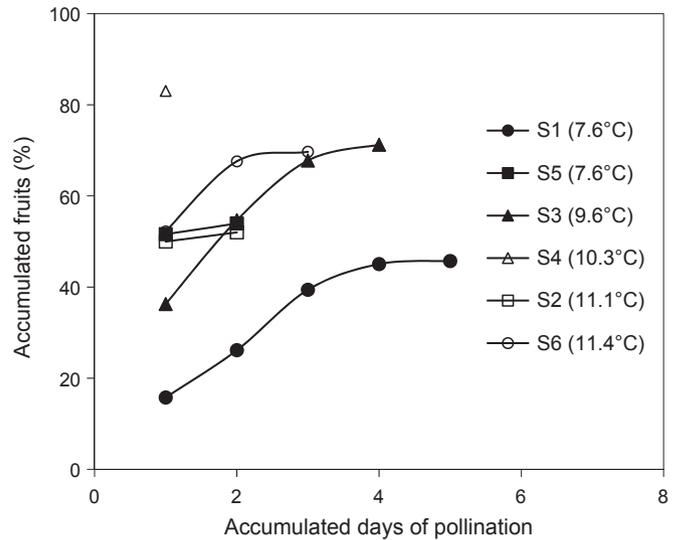


Fig. 5 Accumulated fruit set in *Oxalis compacta* at six high-alpine sites in the central Andes of Chile (sites S1–S6) for the sum of flowers that were successfully pollinated over increasing numbers of days of the flower life-span, expressed as a percentage of the initial recuperated number of flowers that were exposed to open pollination. Mean daily microsite temperature at 15 cm above ground level during the period of flower longevity (FL) observations at each site is given after the site numbers. Closed symbols, the three coldest sites, also with the longest potential FL (see Supporting Information Fig. S2); open symbols, the three warmest sites, also with the shortest potential FL (see Fig. S2). Note that all open-pollinated flowers at site 4 closed on the afternoon of the same day they opened. The curves show the general fruit accumulation tendencies for the sites.

(45.7–71.2%) and three warmest (52.0–83.1%) sites were not very different.

Seed production per fruit across sites varied significantly with flower diameter ($F_{1,9,5} = 5.3$; $P = 0.04$), which is consistent with the results found in the warming experiment. Site variation and temperature did not affect seed production per fruit (the site confidence interval included 0, and all temperature P -values were > 0.5), indicating that when flowers were pollinated they seemed to have received similar pollen loads across sites.

Discussion

Prolonged actual FL under cooler temperatures and/or associated with lower pollination rates at higher altitudes has been reported on several occasions in the alpine zone (Arroyo *et al.*, 1981, 1985; Primack, 1985; Bingham & Orthner, 1998; Utelli & Roy, 2000; Blionis *et al.*, 2001; Blionis & Vokou, 2002; Fabbro & Körner, 2004; Makrodimos *et al.*, 2008) and is often perceived to indicate possible selection on FL. However, more prolonged actual FL at higher altitudes does not necessarily mean that potential FL is also more prolonged. A few reports (Utelli & Roy, 2000; Blionis & Vokou, 2001; Giblin, 2005) have shown potential FL to increase with altitude when large temperature differences are involved. While such a tendency could reflect selection on FL, temperature-driven phenotypic plasticity in FL is also likely to be involved.

Our study provides rigorous evidence that temperature *per se* can produce significant variation in potential FL in an alpine species and that this variation moreover can be found at very local scales. The life-spans of pollinator-excluded, experimentally warmed virgin flowers of *O. compacta* were significantly shorter than under ambient temperature. At six sites, differing in altitude by no more than 370 m, striking thermal variation close to the ground explained a high proportion of the natural variation in potential FL (1–8 d), with potential FL becoming more prolonged as site temperature decreased. Results for potential FL obtained across sites and in the warming experiment moreover were highly consistent. Using mean temperature for the exact days over which pollinator-excluded flowers remained open in the OTCs (13.1°C), according to the regression line for potential FL in Fig. 3, the warmed pollinator-excluded flowers should have lasted 2.0 d, which is very close to the experimental value of 2.1 d.

Possible physiological processes underpinning virgin FL plasticity, as documented in *O. compacta*, are respiration and transpiration. Flower respiration rates increase with flower tissue temperature (Seymour *et al.*, 2009) and cool temperatures associated with lower respiration rates can prolong virgin flower life-spans in cut flowers (Cevallos & Reid, 2000; Çelikel & Reid, 2002). While flower respiration has been determined for several alpine species (Galen *et al.*, 1993; Ashman & Schoen, 1994), a possible link between potential FL, temperature, and respiration rates has yet to be investigated. With regard to transpiration, temperature was inversely correlated with relative humidity at our alpine sites (Fig. S3). Primack (1985) suggested that species growing under hot, dry conditions might have short flower life-spans to avoid heavy transpirational water loss. A related situation whereby high transpirational water loss plastically shortens FL is also conceivable. Both water uptake (Galen *et al.*, 1999) and water loss by flowers can be substantial (Lambrecht, 2013), especially under hot, dry conditions. A possible link between transpiration rates, temperature and potential FL plasticity in general is thus worthwhile investigating in future studies.

Variation in the life-spans of open-pollinated flowers (actual FL) in *O. compacta* was also related to thermal conditions close to the ground, leading to a tendency for coupling between actual and potential FLs. In *O. compacta*, such coupling is a product of the direct effect of temperature on potential FL as it affects flower life-span and an indirect effect of temperature on actual FL manifested through the high temperature sensitivity of high-Andean pollinators and their tendency to forage close to the ground to avoid cool air temperatures. A four-fold difference in pollination rates was found on opposite-facing slopes at 3300 m asl characterized by a 3°C temperature difference close to the ground in *Chaetanthera lycopodioides* (Torres-Díaz *et al.*, 2007), a species that shares *O. compacta*'s low-foraging pollinator species.

Notwithstanding some delay in flower closure following pollination, flower senescence in *O. compacta* was seen to be pollination-triggered. By adjusting the actual FL of a flower for post-pollination senescence lag we were able to pinpoint the interval in each flower life-span over which pollination would have taken place. Using this novel procedure, we found good

evidence not only for amelioration of low pollination rates by prolonged FL, but also for abundant fruit set concentrated early in the shorter potential flower life-spans characteristic of warmer than average sites. Moreover, seed/fruit did not vary across sites, indicating similar pollination intensities for flowers at all sites in spite of slower pollination rates at the colder sites. Our findings for potential FL in high-alpine *O. compacta* fit well with the notion of 'adaptive plasticity' (Scheiner, 1993; see also Nicotra *et al.*, 2010). On the one hand, phenotypic variation in potential FL allows regulation of pollination schedules at an individual plant (and flower) level so as to allow similar levels of fruit set at different alpine sites regardless of site temperature differences. On the other hand, because potential flower life-spans become shortened under temperature conditions that are concomitantly associated with more abundant pollination, plasticity in FL serves to streamline overall flower maintenance costs, which can be high (cf. Ashman & Schoen, 1997). Adaptive plasticity is expected to be selectively favored in highly variable and unpredictable environments (Nicotra *et al.*, 2010; Levin, 2012) of which the alpine habitat, in terms of pollination, is a prime example. In relation to flower maintenance costs, phenotypic plasticity in FL should be especially adaptive in animal-pollinated alpine species, where it has been shown that overall allocation to flowers is much higher than in lowland species (Fabbro & Körner, 2004).

Mean air temperature at Embalse del Yeso, close to our study area, showed an overall increase of *c.* 1.5°C over the interval 1962–2006, suggesting that global warming is already under way in the central Chilean Andes (Quense, 2011). Successful migration to cooler thermal sites and/or persistence of present-day populations under global warming requires sustained seed production. The results of our flower-warming experiment, where the temperature increase obtained in the OTCs was similar to further increases expected over this century, suggest that potential FL in trailing populations of *O. compacta* will become reduced as temperatures continue to warm. However, although individual flowers will be exposed to pollinators for less time, this should be offset by abundant fruit set early in the flower life-span as a result of increased pollinator visitation, in line with what today is seen at the warmest sites occupied by *O. compacta*. Overall, phenotypic plasticity in FL could play a critical role in *O. compacta* under climate warming by allowing trailing populations as well as new populations establishing in the thermally variable alpine landscape to regulate their pollination schedules and use resources for floral maintenance in an optimum way.

Fruit and seed set under global warming will also depend on post-pollination events leading up to fertilization (Hedhly *et al.*, 2009). The high fruit and seed sets we found under supplemental warming in *O. compacta* suggest that a future warmer world is unlikely to have negative effects on pollen germination and fertilization success in this alpine species.

In synthesis, temperature near the ground is a direct driver of potential FL and an indirect driver of actual FL in high-alpine *O. compacta*. Given the high degree of flexibility it provides, temperature-driven phenotypic plasticity in potential FL could turn out to be the principal means whereby reproductive assurance is regulated in outcrossing plant species world-wide in the

cold, yet thermally variable, high-alpine habitat and for that matter in other habitats where stochastic pollination is temperature-related. An appropriate test of this hypothesis for the alpine zone would come from temperature- and phylogenetically controlled multi-species comparisons of potential FL in different altitudinal vegetation belts. In future studies on FL in the alpine zone and elsewhere, thermal conditions around flowers should be taken into account, because even though selection to increase FL is always possible, an effect of ambient temperature on potential FL is expected to be universal. Finally, both phenotypic plasticity in FL and high fruit and seed production under warmer temperatures have important implications for persistence and successful upward migration in self-incompatible alpine species in a warming world.

Acknowledgements

This research was funded by Fondecyt-Chile Grant No. 1085013, Contracts ICM P005-02 and Conicyt PFB-23. Special thanks are extended to the La Parva and Valle Nevado Ski companies for permission to work on their properties and several students at the University of Chile for help with setting up and monitoring the field experiments.

References

- Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B—Biological Sciences* 279: 3843–3852.
- Arroyo MTK, Armesto JJ, Primack RB. 1985. Community studies in pollination ecology in the high temperate Andes of central Chile II. Effect of temperature on visitation rates and pollination possibilities. *Plant Systematics and Evolution* 149: 187–203.
- Arroyo MTK, Armesto JJ, Villagran C. 1981. Plant phenological patterns in the high Andean cordillera of central Chile. *Journal of Ecology* 69: 205–223.
- Arroyo MTK, Humana AM, Domínguez 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: 88–99.
- Arroyo MTK, Munoz MS, Henriquez C, Till-Bottraud I, Perez F. 2006. Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above-tree-line species in the high Andes of Chile. *Acta Oecologica-International Journal of Ecology* 30: 248–257.
- Arroyo MTK, Primack R, Armesto J. 1982. Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. *American Journal of Botany* 69: 82–97.
- Arroyo MTK, Squeo FA. 1987. Experimental detection of anemophily in *Pernettya mucronata* (Ericaceae) in western Patagonia, Chile. *Botanische Jahrbücher für Systematik* 108: 537–546.
- Arroyo MTK, Squeo FA. 1990. Relationship between plant breeding systems and pollination. In: Kawano S, ed. *Biological approaches and evolutionary trends in plants*. Tokyo, Japan: Academic Press, 205–227.
- Ashman T, Schoen D. 1994. How long should flowers live? *Nature* 371: 788–791.
- Ashman T, Schoen D. 1996. Floral longevity: fitness consequences and resource costs. In: Lloyd D, Barrett S, eds. *Floral biology. Studies on floral evolution in animal-pollinated plants*. New York, NY, USA: Chapman & Hall, 112–139.
- Ashman TL, Schoen DJ. 1997. The cost of floral longevity in *Clarkia tembloriensis*: an experimental investigation. *Evolutionary Ecology* 11: 289–300.
- 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: 196–202.
- Bingham RA, Orthner AR. 1998. Efficient pollination of alpine plants. *Nature* 391: 238–239.
- Blionis GJ, Halley JM, Vokou D. 2001. Flowering phenology of *Campanula* on Mt Olympos, Greece. *Ecography* 24: 696–706.
- Blionis GJ, Vokou D. 2001. Pollination ecology of *Campanula* species on Mt Olympos, Greece. *Ecography* 24: 287–297.
- Blionis GJ, Vokou D. 2002. Structural and functional divergence of *Campanula spatulata* subspecies on Mt Olympos (Greece). *Plant Systematics and Evolution* 232: 89–105.
- Castro S, Silveira P, Navarro L. 2008. Effect of pollination on floral longevity and costs of delaying fertilization in the out-crossing *Polygala vayredae* Costa (Polygalaceae). *Annals of Botany* 102: 1043–1048.
- Cavieres LA, Peñaloza A, Kalin Arroyo M. 2000. Altitudinal vegetation belts in the high-Andes of Central Chile (33°S). *Revista Chilena de Historia Natural* 73: 331–334.
- Celikel FG, Reid MS. 2002. Storage temperature affects the quality of cut flowers of Asteraceae. *HortScience* 37: 148–150.
- Cevallos JC, Reid MS. 2000. Effects of temperature on the respiration and vase life of *Narcissus* flowers. *Acta Horticulturae* 517: 335–342.
- Domínguez D. 2010. *Detección de rasgos que compensarían la disminución altitudinal del servicio de polinización comunitaria en una especie heterostilica en la cordillera de los Andes de Chile central*. Santiago, Chile: Universidad de Chile Santiago.
- van Doorn WG. 1997. Effects of pollination on floral attraction and longevity. *Journal of Experimental Botany* 48: 1615–1622.
- Duan YW, Zhang TF, He YP, Liu JQ. 2009. Insect and wind pollination of an alpine biennial *Aconitum gymnandrum* (Ranunculaceae). *Plant Biology (Stuttgart, Germany)* 11: 796–802.
- Duan YW, Zhang TF, Liu JQ. 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: 255–265.
- Fabbro T, Körner C. 2004. Altitudinal differences in flower traits and reproductive allocation. *Flora* 199: 70–81.
- Galen C, Dawson TE, Stanton ML. 1993. Carpels as leaves: meeting the carbon cost of reproduction in an alpine buttercup. *Oecologia* 95: 187–193.
- Galen C, Sherry A, Carroll AB. 1999. Are flowers physiological sinks of faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* 118: 461–470.
- García-Camacho R, Totland O. 2009. Pollen limitation in the alpine: a meta-analysis. *Arctic Antarctic and Alpine Research* 41: 103–111.
- Giblin DE. 2005. Variation in floral longevity between populations of *Campanula rotundifolia* (Campanulaceae) in response to fitness accrual rate manipulation. *American Journal of Botany* 92: 1714–1722.
- Gottfried M, Pauli H, Futschik A, Akhalkatsi M, Barančok P, Benito Alonso JL, Coldea G, Dick J, Erschbamer et al. 2012. Continent-wide response of mountain vegetation to climate change. *Nature Climate Change* 2: 111–115.
- Graae BJ, De Frenne P, Kolb A, Brunet J, Chabrierie O, Verheyen K, Pepin N, Heinken T, Zobel M, Shevtsova A et al. 2011. On the use of weather data in ecological studies along altitudinal and latitudinal gradients. *Oikos* 121: 3–19.
- Gulías J, Traveset A. 2012. Altitudinal variation in the reproductive performance of the Mediterranean shrub *Rhamnus lycioides* L. *Journal of Plant Ecology* 5: 330–336.
- Hartley G, Nell T, Leonard R, Barrett J, Boyle T. 1995. Effect of interior light and temperature on longevity of *Rhpsalidopsis*. *Acta Horticulturae* 405: 164–169.
- Hedhly A, Hormaza JI, Herrero M. 2009. Global warming and sexual plant reproduction. *Trends in Plant Science* 14: 30–36.
- Hörandl E, Dobes C, Suda J, Vit P, Urfus T, Temsch EM, Cosendai AC, Wagner J, Ladinig U. 2011. Apomixis is not prevalent in subnival to nival plants of the European Alps. *Annals of Botany* 108: 381–390.
- Inouye DW. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89: 353–362.
- Inouye DW, Pyke GH. 1988. Pollination biology in the snowy mountains of Australia—comparisons with montane Colorado, USA. *Australian Journal of Ecology* 13: 191–210.

- Kearns CA, Inouye DW. 1993. *Techniques for pollination biologists*. Niwot, CO, USA: University Press of Colorado.
- Kerner von Marilaun A. 1895. *The natural history of plants, their forms, growth, reproduction, and distribution*. New York, NY, USA: Henry Holt.
- Lambrech SC. 2013. Floral water costs and size variation in the highly selfing *Leptosiphon bicolor* (Polemoniaceae). *International Journal of Plant Sciences* 174: 74–84.
- Levin DA. 2012. Mating system shifts on the trailing edge. *Annals of Botany* 109: 613–620.
- Lopez R, Runkle E. 2004. The effect of temperature on leaf and flower development and flower longevity of *Zygopetalum redvale* 'fire kiss'. *HortScience* 39: 1630–1634.
- Lundemo S, Totland Ø. 2007. Within-population spatial variation in pollinator visitation rates, pollen limitation on seed set, and flower longevity in an alpine species. *Acta Oecologica* 32: 262–268.
- Makrodimos N, Blionis GJ, Krigas N, Vokou D. 2008. Flower morphology, phenology and visitor patterns in an alpine community on Mt Olympos, Greece. *Flora* 203: 449–468.
- McCall C, Primack RB. 1992. Influence of flower characteristics, weather, time of day, and season on insect visitation rates in 3 plant-communities. *American Journal of Botany* 79: 434–442.
- Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F *et al.* 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15: 684–692.
- O'Neill SD. 1997. Pollination regulation of flower development. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 547–574.
- Pérez F, Arroyo MTK, Armesto JJ. 2009. Evolution of autonomous selfing accompanies increased specialization in the pollination system of *Schizanthus* (Solanaceae). *American Journal of Botany* 96: 1168–1176.
- Primack R. 1985. Longevity of individual flowers. *Annual Review of Ecology and Systematics* 16: 15–37.
- Quense J. 2011. *Changement climatique et dynamique de la végétation dans les Andes du Chili Central, depuis le milieu du xxe siècle: L'exemple de la Valle de Yerba Loca*. Grenoble, France: Université de Grenoble.
- Rathcke BJ. 2003. Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *American Journal of Botany* 90: 1328–1332.
- Rozzi R, Arroyo MK, Armesto JJ. 1997. Ecological factors affecting gene flow between populations of *Anarthrophyllum cumingii* (Papilionaceae) growing on equatorial- and polar-facing slopes in the Andes of central Chile. *Plant Ecology* 132: 171–179.
- Scheiner SM. 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* 24: 35–68.
- Scherrer D, Körner C. 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography* 38: 406–416.
- Seymour RS, Gibernau M, Pirintsos SA. 2009. Thermogenesis of three species of *Arum* from Crete. *Plant, Cell & Environment* 32: 1467–1476.
- Sierra-Almeida A, Cavieres LA. 2010. Summer freezing resistance decreased in high-elevation plants exposed to experimental warming in the central Chilean Andes. *Oecologia* 163: 267–276.
- Sobrevila C. 1989. Effects of pollen donors on seed formation in *Espeletia schultzii* (Compositae) populations at different altitudes. *Plant Systematics and Evolution* 166: 45–67.
- Steinacher G, Wagner J. 2010. Flower longevity and duration of pistil receptivity in high mountain plants. *Flora* 205: 376–387.
- Stratton DA. 1989. Longevity of individual flowers in a Costa Rican cloud forest: ecological correlated and phylogenetic constraints. *Biotropica* 21: 308–318.
- Torres-Díaz C, Cavieres LA, Munoz-Ramirez C, Arroyo MTK. 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: 455–468.
- Torres-Díaz C, Gómez-González S, Stotz GC, 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: e19497.
- Totland Ø. 1993. Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Canadian Journal of Botany* 71: 1072.
- Totland Ø. 1994. Influence on climate, time of day and season and flower density on insect flower visitation in alpine Norway. *Arctic and Alpine Research* 26: 66–71.
- Totland Ø, Sottocornola M. 2001. Pollen limitation of reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. *American Journal of Botany* 88: 1011–1015.
- Utelli A-B, Roy BA. 2000. Pollinator abundance and behavior on *Aconitum lycoctonum* (Ranunculaceae): an analysis of the quantity and quality components of pollination. *Oikos* 89: 461–470.
- Vesprini JL, Pacini E. 2005. Temperature-dependent floral longevity in two *Helleborus* species. *Plant Systematics and Evolution = Entwicklungsgeschichte und Systematik der Pflanzen* 252: 63–70.
- Yasaka M, Nishiwaki Y, Konno Y. 1998. Plasticity of flower longevity in *Corydalis ambigua*. *Ecological Research* 13: 211–216.
- Zhang ZQ, Li QJ. 2008. Autonomous selfing provides reproductive assurance in an alpine ginger *Roscoea sneideriana* (Zingiberaceae). *Annals of Botany* 102: 531–538.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Location of *Oxalis compacta* sites in the central Andes of Chile (33°S).

Fig. S2 Mean \pm 2 SE for potential flower longevity (FL) measured in pollinator-excluded flowers and for actual FL measured in open-pollinated flowers in *Oxalis compacta* at six high-alpine sites in the central Andes of Chile.

Fig. S3 Relationship between relative humidity and 24-h temperature at 15 cm above ground level in the upper alpine zone, 3100–3470 m above sea level (asl), in the central Andes of Chile for the period in which flower longevity observations were made at six high-alpine sites in *Oxalis compacta*.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.