

Floral allocation at different altitudes in highly autogamous alpine *Chaetanthera euphrasioides* (Asteraceae) in the central Chilean Andes

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Received: 30 July 2012 / Accepted: 20 November 2012 / Published online: 9 December 2012
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Abstract In the alpine life-zone, increasingly slower and unpredictable pollination at the higher altitudes predict an increase in floral investment in strongly outcrossing, pollen-limited biotically pollinated plant species, but not in autonomously self-pollinating species. Plant size, floral and above-ground vegetative biomass and individual capitulum biomass were studied in highly autogamous *Chaetanthera euphrasioides* (DC.) F. Meigen (Asteraceae) at 2,400 m a.s.l. and 3,300–3,400 m a.s.l. in the high Andes of central Chile. Contrary to prediction, altitude had a small positive effect on floral biomass investment and the anisometric relationship between floral investment, and plant size differed at the two altitudes. Individual capitulum size, however, was not affected by altitude. Plastic floral allocation and selection to increase seed production and ameliorate stronger inbreeding at the higher elevations are discussed as possible explanations for the small but unexpected altitudinal increase in floral allocation.

Keywords Alpine · Andes ·
Autonomous self-pollination ·
Chaetanthera euphrasioides · Floral investment

Introduction

In the harsh alpine habitat, all stages of sexual reproduction are constrained by low temperatures and depend on the availability and timely mobilization of adequate resources from the vegetative plant body to reproductive structures. Pollination is the first of the sequence of events leading to seed production and, hence, is of fundamental importance for the success of all later reproductive stages. Flower visitation rates have been found to decline with elevation at the community level (Arroyo et al. 1985; Arroyo and Squeo 1990), among species of the same genus (Blionis and Vokou 2001) and among populations of the same species (Bingham and Orthner 1998; Utelli and Roy 2000). Although lower pollination rates may be compensated by longer flower life-spans (Arroyo et al. 1985; Primack 1985; Stenstöm and Molau 1992; Kudo and Molau 1999; Bingham and Orthner 1998; Utelli and Roy 2000; Arroyo et al. 2006; Steinacher and Wagner 2010; Torres-Díaz et al. 2011), pollen limitation occurs in many arctic and alpine species (García-Camacho and Totland 2009; Torres-Díaz et al. 2011; Arroyo et al. 2012; Torres-Díaz et al. 2011; Fulkerson et al. 2012).

Differences in flower visitation rates across the altitudinal gradient predict altitudinal changes in floral allocation patterns (Fabbro and Körner 2004; Zhao et al. 2006). Even if long-lived high-alpine flowers could eventually be pollinated, exposure for long periods of time prior to pollination increases the probability of flower damage due to predators, herbivores, and extreme weather conditions. Moreover, the longer a flower stays open, the higher are the associated physiological maintenance costs (Schoen and Ashmann 1995). Under these circumstances, a higher flower fraction translating into larger display sizes and more expedient pollination may be expected (Fabbro and Körner 2004; Arroyo et al. 2006). Several studies on alpine species have

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shown that pollinators prefer larger floral displays (Cavieres et al. 1998; Totland 2004; Arroyo et al. 2007, but see Sandring et al. 2007) and showy flowers are commonly found in alpine areas (Peng et al. 2012).

The above predictions fit well for strongly pollen-limited outcrossing and mixed mating species. However, many alpine species are strong selfers (Arroyo and Squeo 1990; Kelley and Latta, 1998; Gomez 2002; Arroyo et al. 2006; Zhi-Qiang and Quing-Jun 2008) and it is well known that autonomous self-pollination is adaptive under impoverished and variable pollination conditions (Kalisz and Vogler 2003) as found in the alpine. If pollinator conditions are a major selective force on altitudinal patterns in flower allocation patterns, in contrast to the situation in strongly outcrossing species, no altitudinal increase in flower allocation is expected in highly autogamous species that are not reliant on biotic pollinator service.

Level of dependence on pollinators is not the only factor predictably involved in shaping floral allocation in the alpine. Partial failure of seed set or incomplete seed ripening is commonplace in high-alpine populations on account of colder temperatures (c.f. Marchant and Roach 1980; Spira and Pollack 1986). In pollen-limited outcrossing species, incomplete seed ripening should further select for expedient pollination and, hence, higher floral allocation to provide sufficient time for seed ripening. To the contrary, in highly autogamous species, reduction in individual flower size is expected at the higher colder elevations to allow more time for seed maturation. The last hypothesis finds an analog in the evolution of increasingly smaller and earlier-maturing highly autogamous flowers over an aridity gradient which increases the time available for seed maturation in relation to water availability (c.f. Mazer et al. 2010). Nevertheless, how these various factors play out ultimately depends on resource availability. Under strong resource limitation, allocation to reproduction in some showy-flowered alpine species has been found to decrease with altitude (Zhao et al. 2006), a tendency that has also been reported in sexually reproducing wind-pollinated *Poa alpina* (Hautier et al. 2009). In relative terms, however, highly selfing species should be able to respond more quickly to resource restrictions because of lack of selection to maintain large floral displays.

In this paper we consider floral (F) and vegetative (V) biomass, as well as individual capitulum biomass at two altitudes in *Chaetanthera euphrasoides* (Asteraceae), a highly autogamous alpine species in the central Chilean Andes (33°S). The main question investigated was: how does reproductive allocation or, more specifically in this case, floral allocation (F/V) vary with altitude? To investigate this question in the context developed here, we took an allometric approach. Recent work using this approach has shed important light on altitudinal patterns in a variety of

reproductive processes in high-elevation ecosystems (Guo et al. 2010a, b). We predict that F/V should not increase with altitude in highly autogamous *C. euphrasoides*.

Methods

Study species

Annual *Chaetanthera euphrasoides* (DC.) F. Meigen is restricted to above tree-line alpine habitats in the mid-latitude mediterranean-climate Andes of South America where it occurs from the subalpine to the upper alpine (1,600–2,200–3,500 m (Arroyo et al. 2006; Davies 2010). Plants have a slender, semi-prostrate habit, with the small capitula (6- to 8-mm diameter) borne terminally on the branches and disposed close to ground level. Flowering takes place over a couple of weeks (Arroyo et al. 1981).

Chaetanthera euphrasoides is highly self-compatible and adapted for autonomous selfing (Arroyo et al. 2006). Seed set levels under autonomous self-pollination, hand-pollination, and open-pollination are statistically indistinguishable and usually >80 % across the altitudinal gradient. In comparison with flower visitation rates in the alpine community at large, levels of visitation can be up to two orders of magnitude lower and are always erratic. Genetic diversity within populations is low. Strongly autogamous *C. euphrasoides* probably originally diverged in the subalpine, to later extend, upward in elevation, where today its high-alpine populations exhibit lower genetic diversity than their lowermost subalpine counterparts (Arroyo et al. 2006).

Sites and sampling

Work was carried out in the Farellones-La Parva-Valle Nevado area adjacent to Santiago (33°S). Mean annual air temperature at 2,400 m a.s.l. is 7.8 °C decreasing to 1.8 °C at 3,400 m (Cavieres et al. 2000). Summers are generally sunny, although increasingly punctuated with afternoon thunder and hail storms at the higher elevations. Three sites were located in the subalpine belt (ca. 2,400 m a.s.l.) and three in the upper alpine belt (ca. 3,300–3,400 m a.s.l.) (see Table 1 for exact site details). Sampling dates corresponded to the height of the flowering season on each site (late December to late January) and well before fruiting had occurred. While fresh and 30 fully flowering plants per site were dissected into their capitulum (including the involucre bracts), stem, leaf and root fractions and the number of capitula per plant recorded. The loose outer foliose photosynthetic capitulum bracts were placed in the leaf fraction. Although the fairly synchronous capitulum development facilitated harvesting the total floral fraction of a plant, we cannot totally discard that the ovules in some capitula had

been recently pollinated, and occasionally some capitula were still immature. Plant material was oven dried at 70 °C for 3 days and the dry weights obtained to the nearest 0.1 mg on an analytical balance (Ohaus). The leaf and stem fractions of each plant were summed to provide a measure of vegetative biomass (V). Although the root material was weighed, we finally opted for discarding root biomass in the main analyses (c.f. Fabbro and Körner 2004), because many of the retrieved roots had been damaged.

Statistical analysis

Altitudinal differences in absolute above-ground plant biomass ($F + V$) and individual capitulum biomass were assessed with nested ANOVA in which altitude was a fixed factor and site was a random grouping factor. Dry weights were not available for each individual capitulum on a plant. Therefore, mean capitulum biomass per plant was obtained by dividing total capitulum biomass by the number of capitula per plant. Data were Log_{10} transformed to meet normality requirements.

To investigate the possible plant size effects on F/V , we used the linearised allometric scaling equation: $\log_{10} Y = \log_{10} a + b \log_{10} X$ (Klinkhamer et al. 1992), where Y is floral biomass (F) and X is above-ground vegetative biomass (V). In a \log_{10} – \log_{10} plot, the exponent b represents the slope and $\log_{10} a$ represents the intercept of the regression of $\log_{10} Y$ on $\log_{10} X$. Under this equation, when $b = 1$, scaling is isometric and F/V is independent of plant size. When b differs from 1, plant size influences F/V either positively ($b > 1$) or negatively ($b < 1$). The null hypothesis of isometry ($b = 1$) was tested with a t test: $(\hat{\beta} - b)/SE_{\hat{\beta}}$, where $\hat{\beta}$ is the estimate based on the sample. Differences in a and b between altitudes were tested with ANCOVA in which V was a covariate and altitude; the interaction between altitude and V were explanatory factors. Altitude was considered as a categorical fixed factor with two levels (high and low) and site pertinence was considered as a random factor. Normality of the residuals was confirmed with the Shapiro–Wilks test. Significance of effects was evaluated with the Wald test. A

significant intercept ($\log_{10} a$) difference indicates an effect of altitude on F/V . A significant interaction between altitude and V indicates that the slopes of the two allometric regression lines (b) differ with altitude. Statistical analyses were undertaken in the nlme package (Pinheiro et al. 2012) in R version 2.15.1.

Results

Total above-ground plant size ($F + V$) varied from 23.4 ± 4.0 (Mean \pm 2SE) to 53.5 ± 12.4 mg (Table 1). There was no significant effect of altitude on plant size ($F_{1,4} = 0.006$, $P = 0.931$). Thus, unlike in many alpine species, there is no overall tendency for plants of *C. euphrasioides* to decrease in size with increasing elevation. Mean individual capitulum biomass varied from 6.5 ± 1.6 to 9.5 ± 2.4 mg (Table 1) and was not affected by altitude ($F_{1,4} = 0.0001$, $P = 0.994$).

Figure 1 shows absolute values of V and F according to altitude. Figure 2 shows the allometric relationship between F and V for *C. euphrasioides* at the two altitudes. Values of b were 0.62 for the high altitude and 0.65 for the low altitude. At both altitudes, we found $b \neq 1$ (high: $t_{171} = -13.3522$, $P < 0.0001$; low: $t_{171} = -12.3492$, $P < 0.001$) indicating that F scales anisometric with V . The tendency at both elevations was decrease in F/V as V increases.

ANCOVA (Table 2) showed that altitude had a significant effect on $\text{Log}_{10} F$ when $\text{Log}_{10} V$ was considered as a covariate ($P = 0.0270$; Table 2). As indicated by the interaction between the covariate and altitude, the slopes of the allometric equations for the two altitudes were not significantly different ($P = 0.2182$; Table 2). These last two results and b values < 1 signify that: (a) high and low altitude populations of *C. euphrasioides* are differentiated in relation to their floral allocation patterns, with a higher level of floral allocation found at the higher altitude; (b) for any given plant size at the two altitudes, floral allocation will always tend to be higher at the higher altitude; (c) in relative terms, the difference in floral allocation becomes more accentuated at the larger plant sizes.

Table 1 Site details, floral (F), vegetative (V) and above-ground biomass ($F + V$) for highly autogamous *Chaetanthera euphrasioides* in the subalpine belt (I–III) and upper alpine belt (IV–VI), Andes of central Chile (33°S). In addition, shown is individual capitulum biomass

Site	Latitude (S)	Longitude (W)	Altitude (masl)	F (mean \pm 2SE) (mg)	V (mean \pm 2SE) (mg)	$F + V$ (mean \pm 2SE) (mg)	Capitulum biomass (mean \pm 2SE) (mg)
I	33° 20' 06.30"	70° 14' 56.73"	3330	20.0 (3.4)	33.5 (9.2)	53.5 (12.4)	8.7 (2.6)
II	33° 19' 36.26"	70° 16' 07.10"	3340	18.1 (2.6)	32.6 (7.1)	50.7 (9.4)	6.5 (1.6)
III	33° 19' 13.62"	70° 14' 28.81"	3410	11.9 (1.9)	11.5 (2.2)	23.4 (4.0)	7.8 (2.0)
IV	33° 22' 07.14"	70° 16' 25.54"	2450	13.8 (2.0)	24.2 (5.3)	38.0 (6.8)	9.5 (2.4)
V	33° 21' 05.04"	70° 18' 31.98"	2450	17.6 (3.7)	32.4 (7.8)	50.0 (11.3)	8.4 (3.3)
VI	33° 21' 50.40"	70° 19' 08.88"	2420	12.3 (2.1)	19.3 (5.2)	31.6 (7.0)	7.2 (2.4)

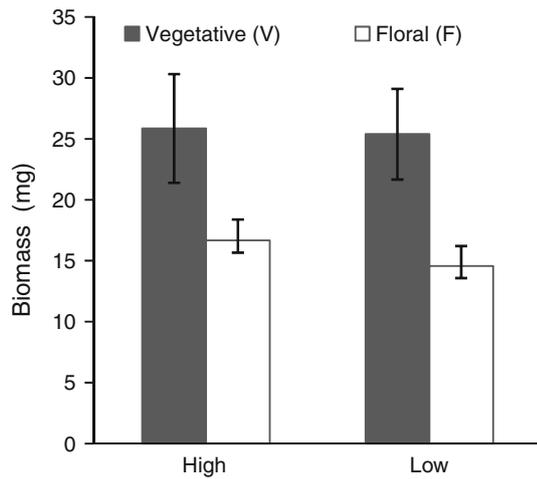


Fig. 1 Vegetative biomass and floral biomass (mean \pm 2SE) in *Chaetanthera euphrasioides* in the central Chilean Andes at two different altitudes. High 3,300–3,400 m a.s.l.; Low 2,400 m a.s.l. Vegetative biomass is the sum of leaf and stem biomass

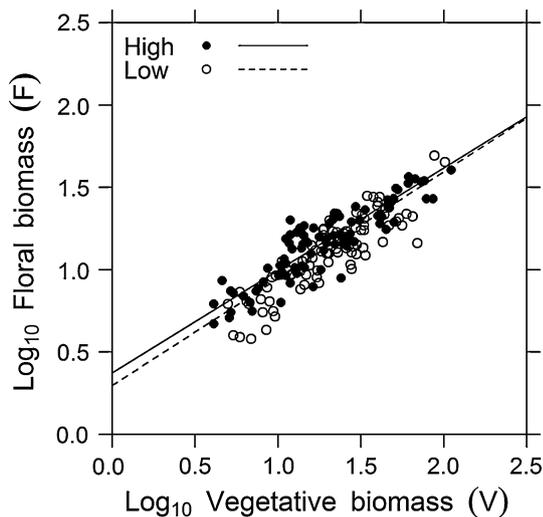


Fig. 2 Relationship between floral biomass (Y) and vegetative biomass (X) at two altitudes in the central Andes of Chile for *C. euphrasioides* (Asteraceae). Low (ca. 2,400 m a.s.l.): $\text{Log}_{10} Y = 0.30 + 0.65\text{Log}_{10} X$; High (ca. 3,300–3,400 m a.s.l.): $\text{Log}_{10} Y = 0.37 + 0.62\text{Log}_{10} X$. Equations are derived from the fixed factors of the ANCOVA model. See text for the allometric coefficient and exponent values

Discussion

To our knowledge, no previous study has focused on altitudinal differences in floral allocation in a highly autogamous alpine plant species. In *C. euphrasioides* absolute above-ground plant size and individual capitulum biomass were invariant over ca. 1,000 m of elevation. Contrary to our original predictions, however, floral investment (F/V) was larger at the higher altitude. While altitude had a small positive effect on F/V , there was no

Table 2 ANCOVA for Log_{10} floral biomass (F) in highly autogamous *Chaetanthera euphrasioides*

	$\text{Log}_{10} V$		Altitude		$\text{Log}_{10} V \times \text{Altitude}$	
	$F_{1,171}$	P	$F_{1,4}$	P	$F_{1,171}$	P
$\text{Log}_{10} F$	579.196	<0.0001	11.624	0.0270	1.528	0.2181

Altitude is a fixed categorical factor. The covariate is log_{10} vegetative biomass (V)

significant interaction between altitude and $\text{Log}_{10} V$. Floral allocation was higher for all plant sizes at the high altitude, with the magnitude of the difference increasing with plant size, in accordance with an altitudinal difference in the allometric relationship between F and V . The altitudinal increase in F/V in *C. euphrasioides* thus is not necessarily independent of the differentiated allometric growth patterns. The statistical model does not allow determining the relative percentage contributions of extrinsic and intrinsic factors. However, because mean plant size did not differ among altitudes, the altitudinal difference is probably more strongly determined by an external factor and less so by intrinsic allometric considerations per se.

The above allocation patterns were based on above-ground vegetative biomass as a metric of plant size. The ratio of above-ground to below-ground biomass tends to be lower in colder biomes (Mokany et al. 2006); Poorter et al. (2012) attributed this tendency to generally lower standing biomass, and plants of the same species often tend to be smaller in high-altitude populations (Körner 2003). This same trend could be expected in *C. euphrasioides*, in which case, not including root biomass (10–18 % of total biomass) could have affected our conclusions. However, there was no significant altitudinal difference in absolute root biomass ($F_{1,4} = 0.255$, $P = 0.640$) as was the case for total above-ground biomass.

We predicted that if anything, further reduction in individual flower (or capitulum in the case of Asteraceae) biomass is expected at the higher altitudes in autogamous alpine species given that the shorter time available for seed maturation is expected to select for faster reproduction allowing more time for seed maturation. Reduction in individual flower size is common in highly autogamous species (Wyatt 1984; Elle and Carney 2003; Elle et al. 2010) also because smaller flowers are often associated with a lower degree of herkogamy (Moeller and Geber 2005; Vallejos-Marín and Barrett 2009) which streamlines assurance of autonomous self-pollen deposition (Armbruster et al. 2002). Nevertheless, altitude per se did not affect individual capitulum biomass in *C. euphrasioides*. This is possibly because the already very high autonomous selfing levels in the lowermost populations of this species (Arroyo et al. 2006) leave little room for additional floral refinements. In addition, although *C. euphrasioides* must

germinate, grow to maturity, flower and fruit over a relatively short growing season compared with lowland species of the genus, the time available for seed maturation is quite long for an alpine species. An important factor here is precocious germination under the snow (Castor 2002) which allows *C. euphrasioides* populations to establish early in the season. As a consequence of the latter, the achenes begin to mature as of early February, for an austral summer season that can still be very warm at 3,400 m a.s.l. as late as mid March.

The unexpected altitudinal difference in floral allocation in *C. euphrasioides* according to our predictions related to breeding system and pollination has several possible explanations. Based on an extensive study of different species and conspecific populations for some species in the genus *Pedicularis* on the eastern Tibetan Plateau, Guo et al. (2012) reached the conclusion that a comparatively lower reproductive allometric exponent is typical for high-altitude species or populations in general. These differences were related to overall differences in average plant size and different biophysical constraints on plants at different altitudes (Guo et al. 2012). In *C. euphrasioides*, the allometric exponent was similar at both altitudes and overall plant size did not change with altitude. Weiner et al. (2009) experimentally demonstrated that different nutrient and water levels, as well as levels of competition, altered the allometric coefficient (a) in annual *Senecio vulgaris* independently of allometric plant size effects. Just as we found in *C. euphrasioides*, the slope of the allometric equation did not change, and the interaction term was not significant. In the *S. vulgaris* experiment, the less-favorable conditions resulted in lower efficiency in the conversion of vegetative biomass into reproductive biomass such that for any given plant size, reproductive investment became lower. *C. euphrasioides* grows in a mediterranean-climate alpine area characterized by much higher water stress at the lower altitudes (Cavieres et al. 2006). Therefore, the somewhat larger floral investment at the higher altitude in annual *C. euphrasioides* might simply represent a plastic response to greater water availability at that altitude.

Nevertheless, such higher floral allocation could have arisen in relation to extrinsic factors other than pollination, such as compensation for higher seed abortion (and perhaps lower germination success) at the higher elevation. These last factors should be especially important in high-elevation annuals where populations must be re-established yearly. On the other hand, we know that levels of genetic diversity in high-altitude populations of *C. euphrasioides* are low in relation to those in low-altitude populations (Arroyo et al. 2006). This last situation might favor some increased floral allocation as a means of maintaining sporadic cross-pollination so as to avoid very high levels of inbreeding in those populations.

Conclusions

Floral investment in highly autogamous *C. euphrasioides* was not constant at different altitudes as was predicted from a breeding system and pollination perspective. That individual capitulum biomass does not decrease with altitude suggests that selection to increase the efficiency of self-pollination and the time available for seed maturation at the higher altitudes has not occurred in this highly autogamous species. Prior to evaluating the hypotheses that increased floral investment at higher altitudes in *C. euphrasioides* is a response to selection for higher seed set or for counteracting inbreeding, reciprocal transplant experiments are needed to discard possible plastic allometric responses to different environmental conditions at the high and low altitudes.

Acknowledgments This work 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. Anonymous reviewers are thanked for a number of suggestions that have contributed to improving the paper.

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