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Selection on flowering time in Mediterranean high-mountain plants under global warming

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Abstract Under climate warming, plants will undergo novel selective pressures to adjust reproductive timing. Adjustment between reproductive phenology and environment is expected to be higher in arctic and alpine habitats because the growing season is considerably short. As early- and late-flowering species reproduce under very different environmental conditions, selective pressures on flowering phenology and potential effects of climate change are likely to differ between them. However, there is no agreement on the magnitude of the benefits and costs of early- vs. late-flowering species under a global warming scenario. In spite of its relevance, phenotypic selection on flowering phenology has rarely been explored in alpine plants and never in Mediterranean high mountain species, where selective pressures are very different due to the summer drought imposed over the short growth season. We hypothesized that late-flowering plants in Mediterranean mountains should present stronger selective pressures towards early onset of reproduction than early-flowering species, because less water is available in the soil as growing season progresses. We performed selection analyses on flowering onset and duration in two high mountain species of contrasting phenology. Since phenotypic selection can be highly context-dependent, we studied several populations of each species for 2 years, covering their local altitudinal ranges and their different microhabitats. Surrogates of biotic selective agents, like fruitset for pollinators and flower and fruit loss for flower and seed predators, were included in the analysis. Differences between the early- and the late-flowering species were less than expected. A consistent negative correlational selection of flowering onset and duration was found affecting plant fitness, i.e., plants that bloomed earlier flowered for longer periods improving plant fitness. Nevertheless, the late-flowering species may

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experience higher risks under climate warming because in extremely warm and dry years the earlier season does not bring about a longer flowering duration due to summer drought.

Keywords Flowering phenology · Phenotypic selection · Alpine plants · Altitudinal gradients

Introduction

Phenology evolves to match environmental conditions and maximize individual fitness (Stanton et al. 2000; Donohue 2005). By adjusting to the abiotic and biotic environment where growth and reproduction take place, flowering phenology can dramatically affect the selective environment to which plants are exposed, with the resulting consequences on plant fitness. An adequate match between phenology and environment is especially critical in high-mountain plants because the growing season is usually very short and unpredictable (Stinson 2004; Alatalo and Totland 2003; Totland 1999; Hegland et al. 2009).

The climate in high mountains is warming up at an exceptionally high rate (Nogués-Bravo et al. 2007). This temperature increase primarily affects snowmelt and, consequently, the onset and length of the growing season (Beniston et al. 1997; Dye 2002; Inouye 2008). In fact, high-mountain plants are among those organisms for which warming is thought to be especially critical (Theurillat and Guisan 2001; Inouye et al. 2002; Thuiller et al. 2005). Upward and poleward range shifts (Grabherr et al. 1994; Klanderud and Birks 2003; Kelly and Goulden 2008) together with phenotypic plasticity and even local adaptation to novel conditions seem to be the only available alternatives to ensure long-term persistence (Jump and Peñuelas 2005; Giménez-Benavides et al. 2007b). Phenological shifts in high-mountain plants have been documented in both observational surveys (Inouye et al. 2002; Kudo and Hirao 2006; Inouye 2008) and manipulative warming experiments (Price and Waser 1998; Dunne et al. 2003). However, to our knowledge, it is not known whether these phenological shifts are the result of evolutionary changes or simple plastic responses to warming (Bradshaw and Holzapfel 2006; Burgess et al. 2007). Moreover, an assessment of the evolutionary potential of phenological shifts in high-mountain plants to face current climate change challenges is long due.

Most field observations on high-mountain plants suggest that an early-flowering strategy is always advantageous regardless of flowering timing (Galen and Stanton 1993; Kudo 1991; Stenström and Molau 1992; Totland 1994, 1997; Dunne et al. 2003). However, there is no agreement on the relative benefits of early-flowering in early- vs. late- flowering species under a global warming scenario. Some authors sustain that early-flowering species, so-called pollen-riskers, will not have a significant advantage because flowering too early may affect reproductive success due to pollinator scarcity (Molau 1993; Alatalo and Totland 2003; Totland 1997; Kudo and Hirao 2006). Moreover, flowering earlier can expose plants to a greater risk of early-summer frosts and subsequent bud mortality (Inouye 2008 and references therein). On the other hand, late-flowering species are usually exposed to risks during seed ripening due to early snow or frost events in late summer/early autumn (Molau 1993; Galen and Stanton 1995), the so-called seed-riskers. These plants may be favoured by warming scenarios because of the extended growing season (Molau 1993; Alatalo and Totland 2003; Molau et al. 2005). However, these hypotheses were mainly made on observations of arctic and temperate alpine species. The growing season may not extend in a warming world in high-mountain plants of Mediterranean climate regions if summer drought also increases (Giménez-Benavides et al. 2007a). Climate trend projections for Mediterranean mountains predict greater warming rates than those reported for other mountain regions, together with lower precipitation mainly during spring (Nogués-Bravo et al. 2008). We therefore hypothesized that more intense summer droughts in Mediterranean high mountains might impose novel selective scenarios not considered in other mountain areas. Our conceptual scheme is represented briefly in Fig. 1. We expected that early-flowering plants in Mediterranean high mountains would benefit from milder temperatures and the snowmelt water of spring - early summer, while late-flowering plants would face more stressful conditions imposed by soil water shortage and higher temperatures. In a warming scenario in Mediterranean mountains, earlier snowmelt would result in an earlier onset of both the growing season and summer drought. Thus, we hypothesized that both early- and late- flowering species would show a selective pressure towards early blooming. More specifically, we hypothesized that late-flowering species would present stronger selective pressures towards early onset of reproduction, because some individuals may not be able to flower before the arrival of summer drought.

To explore this hypothesis, we selected two co-occurring Mediterranean high-mountain specialists with contrasting flowering strategies: *Armeria caespitosa*, one of the first bloomers in the orophyllous cryophilic pastures above the treeline (pollen-risk species), and *Silene ciliata*, an extremely late-flowering plant (seed-risk species). Concerning photoperiod control, previous studies (Giménez-Benavides et al. 2007a; García-Camacho and Escudero 2009) suggest that *A. caespitosa* is a neutral plant that flowers immediately after snowmelt, whereas *S. ciliata* may be considered a genuine long-day (LD) plant that flowers only after a particular day length is reached, as recorded for other *Silene* species (Lang 1957; Talon and Zeewaart 1990; Donnison and Francis 1994). Each species initiates its reproductive cycle under very different environmental conditions, so that potential

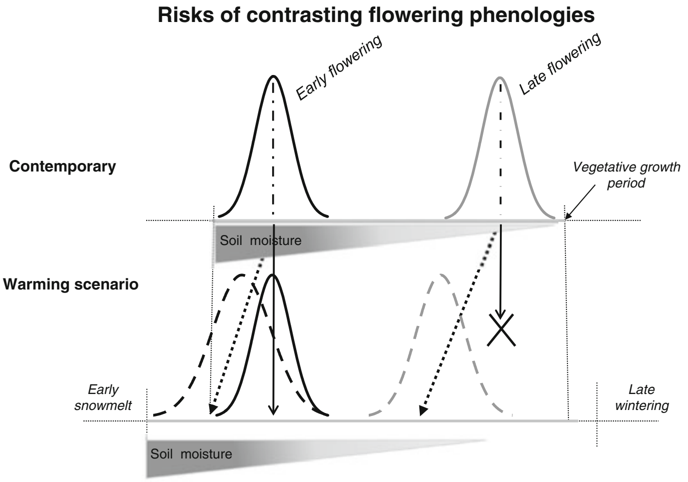

Fig. 1 Conceptual scheme showing the potential risks of early vs. late flowering species under global warming in Mediterranean high mountains. In a warming scenario, the vegetative growth period is likely to increase due to early snowmelt time and late wintering. As summer precipitation is really scarce in this climate, soil moisture mainly depends on the supply of snowmelting water, which is progressively reduced as the season advances and becomes limitant earlier. In this context, early-flowering species can keep (*solid arrow*) or anticipate (*broken arrow*) their reproductive timing (represented as a *flowering curve*), increasing flowering duration (flowering *curve width*) in the latter. However, late-flowering species are likely forced to shift the flowering phenology to avoid the most stressful part of the season

effects of climate warming are likely to differ (Molau 1993; Totland 1997; Alatalo and Totland 2003). Hence, these species constitute ideal model systems to test our working hypothesis.

We used an altitudinal gradient approach to evaluate how phenotypic selection on flowering time may be modulated by climate conditions, because study sites along altitudinal gradients may be a good proxy of how plants respond to warming scenarios (Körner 2007). Since pollinators and predators can act as selective agents on flowering time and exert opposing or fluctuating selection on flowering phenology (Elzinga et al. 2007; Ehrlén and Münzbergová 2009; Sandring and Ågren 2009; Gómez et al. 2009), we identified and included several sequential selective episodes in the reproductive cycle, from flower production and predation to fruit ripening and predation. With this altitudinal gradient approach and the highly contrasting flowering times observed in these two species, we aimed to assess: (1) whether shifts in flowering phenology may confer advantages to high-mountain plants, (2) whether differences in the strength and direction of selective pressures exist in early vs. late flowering species, (3) whether selective forces differ among altitudes within species, and (4) whether conflicting selection exists within populations when many potential selective agents (pollinators, predators) act simultaneously on the same phenological traits.

Materials and methods

Species and study area

Silene ciliata Poiret is a perennial cushion plant which inhabits the main Mediterranean mountain ranges in the northern half of the Mediterranean Basin reaching its southern latitudinal limit in the Iberian Central Range. Flowering stems reach 15 cm in height and bear 1–5 flowers. Although the species is self-compatible, it requires pollen vectors to produce a full seed set (Giménez-Benavides et al. 2008). A total of 11 insect species were recorded visiting *S. ciliata* flowers: hoverflies (Syrphidae), hawkmoths (Sphingidae), and butterflies and moths (Lepidoptera) (Giménez-Benavides et al. 2007c). Flowers are mainly predated by the blister beetle *Mylabris sobrina* Graëlls, 1849 (Coleoptera: Meloidae), and occasionally by nectar-thieving, florivorous ants (Hymenoptera: Formicidae) (Giménez-Benavides et al., 2008). Pre-dispersed seeds are consumed by the moth *Hadena consparcatoides* (Schawerda 1928) (Lepidoptera: Noctuidae), and rarely by weevils (Coleoptera: Curculionidae) (Giménez-Benavides et al. 2007c, 2008). *H. consparcatoides* acts as a nursery pollinator of *S. ciliata* (i.e. flowers are used for oviposition and as brood sites for the pollinator's developing larvae). Therefore, this moth species can act as a mutualist or parasite depending on the trade-off between pollination services and seed predation losses and the presence of effective co-pollinators (Giménez-Benavides et al. 2007c).

Armeria caespitosa (Gómez Ortega) Boiss. *in* DC. is a high-mountain perennial dwarf cushion plant, endemic to the eastern Iberian Central Range, ocurring from 1,700 to 2,430 m. It grows in two interspersed but contrasting microhabitats: crevasses and ledges of granite and gneiss outcrops (hereafter, rocky microhabitat), and dry cryophilic *Festuca curvifolia* fellfields (hereafter, pasture microhabitat). It presents pink flowers grouped in short-scaped flowerheads. As in most species of the genus, *A. caespitosa* has a dimorphic self-incompatibility system (see García-Camacho et al. 2009 for more details). Flowers are visited by several species of hoverflies (Syrphidae), butterflies (Lepidoptera), honey bees and bumblebees (Hymenoptera, Apidae). Pre-dispersal seed predation is produced by larvae of *Sibinia* weevils (Coleoptera, Curculionidae), but there is no evidence of flower predation (García-Camacho, personal observation).

The study area was located in the Sierra de Guadarrama (Madrid, 40°N, 3°W), a W–E running mountain range characterized by a Mediterranean-type climate with an intense dry season from June to September (less than 10% of total annual rainfall) (Giménez-Benavides et al. 2007a). Vegetation in the tree line (1,900–2,000 m a.s.l.) is dominated by a low dense stand of *Pinus sylvestris* interspersed in a matrix of shrub species (*Cytisus oromediterraneus* and *Juniperus communis* subsp. *alpina*) and perennial grasses dominated by *Festuca curvifolia*. The *Cytisus-Juniperus* shrub formation is progressively replaced in altitude by the patchy xerophytic pasture dominated by *F. curvifolia* and several endemic perennial high-mountain cushion plants in the summit flat areas and crests (from 2,100–2,200 to 2,430 m.a.s.l.).

We selected populations of each species covering the local species' altitudinal range. Special care was taken to include the lowest and highest populations, considering only functional populations and non-isolated individuals, as well as the different main microhabitats colonized by each species. Therefore, we selected three populations of *S. ciliata* growing in a pasture microhabitat (at 1,976, 2,256, and 2,428 m a.s.l., hereafter low, medium and high populations, Table S1). Previous demographic study showed that long-term persistence at the low population is critically reduced (Giménez-Benavides et al. 2010). For *A. caespitosa*, four populations were selected, two growing in a rocky microhabitat (at 1,750 and 2,365 m a.s.l., hereafter low rock and high rock) and two in a pasture microhabitat (at 2,000 and 2,365 m a.s.l., hereafter low pasture and high pasture), corresponding to the lowest and highest altitudinal boundaries of each microhabitat (Table S1). Approximate snowmelt date at each site was estimated from landscape digital images taken at 10-day intervals.

Data collection and determination of selective episodes

We tagged 60–160 plants in each population before the onset of flowering (see Giménez-Benavides et al. 2007a and García-Camacho and Escudero 2009 for further details on sample sizes), and tracked each plant individually every 5–8 days from flowering to seed dispersal. At each census, we recorded the number of open flowers and mature fruits, as well as the number of predated flowers and fruits. The study was performed for two consecutive years for each species, from 2002–2003 for *S. ciliata* and from 2004–2005 for *A. caespitosa*. (see Giménez-Benavides et al. 2007a and García-Camacho and Escudero 2009 for further details). Hence, we analyzed six different selective scenarios for *S. ciliata* (3 altitudes × 2 years), and eight selective scenarios for *A. caespitosa* (2 altitudes × 2 microhabitats × 2 years). The comparison between the two species may be somewhat limited because they were not monitored in the same years, due to logistic restrictions. However, this is compensated by the fact that both species included an average year (2002 and 2004) and an extreme year (2003 and 2005) in terms of high temperature and low precipitation in their study.

The following phenotypic traits were recorded for each individual: *flower number* (total flower production over the entire reproductive season)*; flowering onset* (number of days from 1 day before the first flowering date in the population to the first flowering date in the individual), and *flowering duration* (number of days the plant remained in bloom). Previous studies showed that these phenological variables are good predictors of female reproductive success in both species (Giménez-Benavides et al. 2007a, García-Camacho and Escudero 2009). Traits were standardized separately for each population and year.

Plant fitness was estimated using individual fruit production in each season as a proxy. We identified three sequential selective episodes (k_{th}) in the life-history stages of the species' reproductive cycles, from initial flower production to fruit production. The fitness components (w'_k) associated to these episodes were: $w'_1 =$ proportion of flowers surviving predation by blister beetles and nectar-thieving, florivorous ants (only for *S. ciliata*); $w'_2 =$ proportion of non-predated flowers developing a mature fruit (fruit set); and $w'_3 =$ proportion of mature fruits surviving predispersal seed-predation by moths and weevils. Each fitness component was relativized as the individual absolute fitness divided by the average fitness in the population (Wade and Kalisz 1989). Only individuals reaching a given transition were included in the calculation of the corresponding fitness component, so w'_ks are independent (Arnold and Wade 1984a, b). The independent method was used rather than the additive one, because the immediate aim was to determine the selection occurring at each stage independent of selection at other stages, as well as to explore potential evolutionary trade-offs (Koenig et al. 1991).

Statistical analysis

Selection during each episode was estimated by selection differentials and gradients. Selection differentials indicate the strength and direction of the total selection (s) acting on each phenotypic trait during an episode, and were estimated by the covariance between that trait and the fitness values (Lande and Arnold 1983; Arnold and Wade 1984a, b). Selection gradients reveal the strength and direction of the direct selection (β) acting on each trait during a selective episode, independent of the other traits included in the model (Lande and Arnold 1983). Selection gradients were estimated from the standardized partial regression coefficients of linear multiple regression analyses using each fitness component as the response variable and standardized trait values as explanatory variables. Selection differentials and gradients were estimated using SAS v.9.0 (SAS Institute 1996). Statistical significance of selection differentials was obtained through permutation tests with 1,000 iterations using a script in R statistical package (R Development Core Team 2010). To quantify non-linear and correlational selection, we then performed new multiple regression models including quadratic terms and/or interaction terms. However, in most scenarios the R^2-values of the selection models increased only marginally, suggesting that selection was predominantly directional. We, therefore, only report linear selection gradients with main effects.

Structural equation modeling (SEM) with the independent fitness components was used to detect the relative importance of each selective episode and to clarify the causes of selection occurring throughout the reproductive cycle (Conner 1996; Scheiner et al. 2000; Gómez 2008). An a priori saturated SEM model (Fig. 2) was constructed connecting all plant phenological traits to each independent fitness component, and these components to our surrogate of maternal fitness (i.e., fruit production). We also included flower number as a variable in our SEM models to discriminate between direct and indirect selection through covariation with flower number (Gómez 2008; Ehrlén and Münzbergová 2009). We then performed several alternative nested models, in which some of the causal paths of the saturated model were sequentially constrained to zero (i.e., the least significant paths, those reaching the highest p-values in all selective scenarios, see Table S2). The models were solved through an iterative process that used generalized least squares (GLS) as the discrepancy function, as this procedure is known to provide additional precision in parameter estimates when sample size is small (Henning Olson et al. 2000). We also used GLS estimation on the variance–covariance matrix to test the goodness of fit of the models and to calculate the Akaike Information Criterion (AIC). In order to select the best-fitting model, we chose the one with the lowest chi-square and AIC values (Shipley 2000; Grace 2006). Significance of individual path coefficients was estimated from 95% confidence intervals by bootstrapping on 2000 random samples. SEMs were performed with AMOS v.5.0.1 (Arbuckle 2003).

We calculated the effects of direct, indirect and total selection of each phenological trait on the fitness components from the selected best-fitting models. Direct selection (β', to differentiate it from selection gradients) is any forward connection between a trait and fitness, and is equivalent in path analysis terminology to the sum of direct and indirect (mediated) effects. Significance of direct selection was also computed by bootstrapping. Indirect selection is any backward connection between a trait and fitness, including correlational effects. Finally, total selection (s') is the sum of direct and indirect selection (Scheiner et al. 2000). Total selection (s') is somewhat equivalent to the selection differentials (s), so we can use path analysis to partial out the selection differential into direct selection (direct + indirect effects) and indirect selection (often called non-causal effects). For further details see Scheiner et al. (2000).

Results

Flowering phenology was markedly different between the two species. Flowering onset occurred in May–June in *A. caespitosa*, and in July–August in *S. ciliata* (Table S1). *Armeria caespitosa* came into bloom soon after snowmelt in all populations, behaving as a typical pollen-risk bloomer. In contrast, *S. ciliata* came into bloom a month after snowmelt or later (Table S1). Our data suggest that *S. ciliata* never flowers before the end of June, even in years when snowmelt is very early (see snowmelt dates for 2003 in Table S1).

Correlations between phenotypic traits and fitness components

Flowering onset and duration in *S. ciliata* were negatively correlated in most cases, but this correlation varied from significant to non-significant depending on the year (Table S3). A similar pattern was found in *A. caespitosa.* Flowering onset and duration were negatively correlated at the lower altitudinal range for both years and microhabitats, while this correlation varied from significant to non-significant at the higher limit.

Few significant correlations were found between fitness components. In *S. ciliata,* w'_1 was positively correlated to w'_2 at the low and high populations in 2002. However, no correlation was found in extremely dry 2003. In *A. caespitosa,* a positive correlation was only found between w'_2 and w'_3 in the rock microhabitat at the higher altitude in 2004 (Table S3).

Selection differentials and gradients

Selection analyses showed that flowering onset and duration were selected during different episodes of the reproductive process, although not consistently between years, altitudes or microhabitats. In both *S. ciliata* and *A. caespitosa*, the most consistently selected trait was flowering onset at the selective episode corresponding to w'_2 (non-predated flowers setting fruit) (Table 1). In contrast, at w'_1 (flowers escaping predation) and w'_3 (fruits escaping predation) selective episodes, selection differentials and gradients were significantly different from zero in very few selective scenarios.

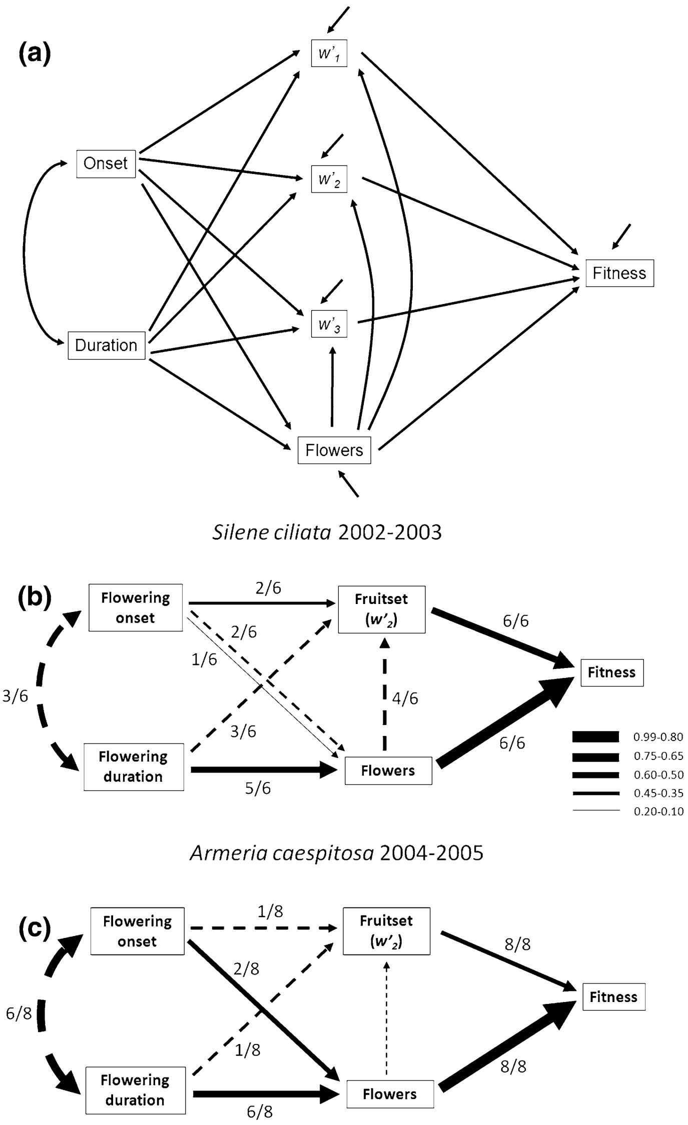

Fig. 2 Structural equation models showing the causal relationships between phenological traits, partial components of fitness and plant fitness. **a** Initial saturated model. Summary of results of the most parsimoious model for *Silene ciliata* (**b**) and for *Armeria caespitosa* (**c**). The graphs in **b** and **c** show a synthetic view of the significant paths resulting at the six and eight selective scenarios in *Silene ciliata* and *Armeria caespitosa,* respectively. *Solid arrows* denote positive effects, *broken arrows* denote negative effects and *arrow widths* are proportional to the average of significant standardized path coefficients across scenarios. Fractions next to *arrows* denote the frequency of selective scenarios where that path was significant (i.e. 3/6 = significant in three out of six scenarios). For complete path diagrams of each scenario see appendix

Plant fitness (fruit number) showed significant selection for phenological traits in many scenarios. Flowering onset was negatively selected in half the selective scenarios of each species, whereas flowering duration was positively selected in all scenarios in *S. ciliata* and

Table 1 Selection differentials (s) and gradients (β) (± 1 standard error) on each phenological trait through each independent fitness component (w'_k) and the plant fitness (fruit number) for (a) *S. ciliata* and (b) *A. caespitosa*

Traits	Selection	w'_1	w'_2	w'_3	Fitness
(a) Silene ciliata					
2002/Low					
Onset	Differential	0.012	**0.176**	0.032	***−0.301***
	Gradient	0.01 ± 0.01	**0.35 ± 0.11****	**0.07 ± 0.03***	0.20 ± 0.10
Duration	Differential	−0.003	0.062	0.004	***0.569***
	Gradient	0.01 ± 0.01	0.23 ± 0.12	0.04 ± 0.03	0.03 ± 0.11
Flowers	Differential	−0.009	0.065	0.009	**1.234**
	Gradient	−0.01 ± 0.01	0.07 ± 0.10	0.01 ± 0.02	**1.29 ± 0.10*****
2002/Medium					
Onset	Differential	0.004	***0.210***	0.014	***−0.267***
	Gradient	−0.00 ± 0.01	0.13 ± 0.14	0.01 ± 0.03	0.12 ± 0.14
Duration	Differential	−0.010	−0.138	0.007	***0.493***
	Gradient	−0.01 ± 0.01	0.00 ± 0.14	0.02 ± 0.03	0.17 ± 0.15
Flowers	Differential	−0.010	***−0.269***	−0.029	**0.859**
	Gradient	−0.01 ± 0.01	−0.22 ± 0.14	−0.02 ± 0.02	**0.83 ± 0.15*****
2002/High					
Onset	Differential	−0.003	0.038	−0.007	***−0.286***
	Gradient	−0.01 ± 0.01	−0.04 ± 0.09	−0.01 ± 0.01	0.01 ± 0.09
Duration	Differential	−0.003	***−0.151***	−0.007	***0.461***
	Gradient	0.01 ± 0.01	−0.09 ± 0.11	−0.00 ± 0.01	−0.14 ± 0.10
Flowers	Differential	**−0.012**	***−0.162***	0.003	**0.860**
	Gradient	**−0.02 ± 0.01****	−0.12 ± 0.11	0.00 ± 0.01	**0.95 ± 0.10*****
2003/Low					
Onset	Differential	***−0.050***	−0.139	−0.031	−0.319
	Gradient	−0.04 ± 0.02	−0.19 ± 0.25	−0.04 ± 0.04	−0.11 ± 0.19
Duration	Differential	0.012	0.080	−0.018	***0.710***
	Gradient	−0.02 ± 0.03	0.10 ± 0.24	−0.04 ± 0.04	0.04 ± 0.23
Flowers	Differential	***0.041***	0.008	0.044	**1.193**
	Gradient	0.04 ± 0.03	−0.06 ± 0.24	0.05 ± 0.04	**1.15 ± 0.23*****
2003/Medium					
Onset	Differential	−0.009	−0.105	−0.005	−0.038
	Gradient	−0.01 ± 0.01	−0.11 ± 0.10	−0.01 ± 0.01	−0.02 ± 0.10
Duration	Differential	−0.007	−0.047	***−0.023***	***0.474***
	Gradient	−0.01 ± 0.01	−0.01 ± 0.11	−0.02 ± 0.01	0.14 ± 0.11
Flowers	Differential	−0.003	−0.113	***−0.023***	**0.770**
	Gradient	0.00 ± 0.01	−0.11 ± 0.11	−0.01 ± 0.01	**0.70 ± 0.11*****
2003/High					
Onset	Differential	−0.002	**0.249**	−0.007	0.138
	Gradient	−0.00 ± 0.00	**0.24 ± 0.07*****	−0.01 ± 0.01	0.11 ± 0.08
Duration	Differential	−0.002	−0.025	0.012	**0.451**
	Gradient	−0.00 ± 0.00	0.06 ± 0.08	0.01 ± 0.01	**0.26 ± 0.09****

Table 1 continued

Traits	Selection	w'_1	w'_2	w'_3	Fitness
Flowers	Differential	−0.001	**−0.183**	0.008	**0.470**
	Gradient	0.00 ± 0.00	**−0.21 ± 0.08****	−0.00 ± 0.01	**0.33 ± 0.09*****

Traits	Selection	w'_2	w'_3	Fitness
(b) Armeria caespitosa				
2004/Low/Rock				
Onset	Differential	0.116	−0.013	***−0.285***
	Gradient	0.02 ± 0.19	−0.00 ± 0.04	−0.13 ± 0.22
Duration	Differential	−0.141	0.013	0.244
	Gradient	−0.13 ± 0.19	0.00 ± 0.04	−0.27 ± 0.22
Flowers	Differential	−0.043	0.021	**0.924**
	Gradient	0.02 ± 0.14	0.02 ± 0.03	**0.99 ± 0.15*****
2004/Low/Pasture				
Onset	Differential	0.060	***−0.189***	**−0.123**
	Gradient	0.06 ± 0.05	0.07 ± 0.10	**0.28 ± 0.12***
Duration	Differential	−0.042	**0.338**	**0.618**
	Gradient	−0.01 ± 0.05	**0.42 ± 0.11*****	**0.49 ± 0.13*****
Flowers	Differential	−0.009	0.084	**0.928**
	Gradient	0.00 ± 0.04	−0.07 ± 0.09	**0.77 ± 0.10*****
2004/High/Rock				
Onset	Differential	***0.142***	−0.004	0.164
	Gradient	0.11 ± 0.07	−0.00 ± 0.00	0.03 ± 0.07
Duration	Differential	***−0.122***	0.004	0.135
	Gradient	−0.04 ± 0.07	0.00 ± 0.01	−0.09 ± 0.07
Flowers	Differential	0.013	0.001	**1.279**
	Gradient	0.01 ± 0.04	0.00 ± 0.00	**1.29 ± 0.04*****
2004/High/Pasture				
Onset	Differential	**−0.069**	−0.001	−0.083
	Gradient	**−0.07 ± 0.03***	−0.00 ± 0.00	**−0.08 ± 0.04***
Duration	Differential	***−0.085***	0.000	***0.231***
	Gradient	−0.06 ± 0.03	0.00 ± 0.00	−0.05 ± 0.05
Flowers	Differential	***−0.086***	−0.001	**0.628**
	Gradient	−0.06 ± 0.03	−0.00 ± 0.00	**0.65 ± 0.05*****
2005/Low/Rock				
Onset	Differential	−0.016	−0.017	***−0.319***
	Gradient	−0.03 ± 0.13	−0.01 ± 0.01	−0.11 ± 0.14
Duration	Differential	−0.021	***0.013***	***0.626***
	Gradient	0.03 ± 0.15	0.00 ± 0.01	−0.15 ± 0.16
Flowers	Differential	−0.099	0.005	**1.233**
	Gradient	−0.12 ± 0.12	−0.00 ± 0.01	**1.29 ± 0.13*****
2005/Low/Pasture				
Onset	Differential	0.009	***0.038***	***−0.258***
	Gradient	0.08 ± 0.09	0.03 ± 0.02	0.04 ± 0.07

Table 1 continued

Traits	Selection	w'_2	w'_3	Fitness
Duration	Differential	0.042	−0.024	***0.240***
	Gradient	0.03 ± 0.09	−0.01 ± 0.02	0.00 ± 0.07
Flowers	Differential	**0.167**	−0.003	**0.992**
	Gradient	**0.18 ± 0.08***	0.01 ± 0.02	**1.00 ± 0.07*****
2005/High/Rock				
Onset	Differential	**−0.169**	−0.009	−0.101
	Gradient	**−0.18 ± 0.09***	−0.01 ± 0.02	−0.05 ± 0.09
Duration	Differential	−0.035	0.008	***0.603***
	Gradient	−0.07 ± 0.10	0.00 ± 0.02	−0.07 ± 0.10
Flowers	Differential	0.003	***0.016***	**1.406**
	Gradient	0.03 ± 0.10	0.01 ± 0.02	**1.44 ± 0.10*****
2005/High/Pasture				
Onset	Differential	−0.027	−0.016	−0.110
	Gradient	0.00 ± 0.07	−0.01 ± 0.01	−0.04 ± 0.07
Duration	Differential	0.069	0.014	***0.518***
	Gradient	0.07 ± 0.08	0.01 ± 0.01	0.07 ± 0.08
Flowers	Differential	0.031	0.006	**0.917**
	Gradient	−0.00 ± 0.07	0.00 ± 0.01	**0.88 ± 0.07*****

In selection differentials bold figures denote significant trait-fitness covariance ($p < 0.05$) and italic figures denote indirect rather than direct selection (see text)

In selection gradients * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$. Trait names in boldface indicate potential conflicting selection (significant opposite-selection during different episodes of selection)

in six of eight scenarios in *A. caespitosa* (Table 1). Comparison of the significance and strength of selection differentials and gradients on plant fitness showed that selection was indirect in most cases (Table 1). Flowering onset was only under direct selection in one selective scenario of *A. caespitosa* (2004, low pasture), and flowering duration was only under direct selection in one case in both *S. ciliata* (2003, high population) and *A. caespitosa* (2004, low pasture). Finally, flower number greatly determined plant fitness in all scenarios, but was weakly selected for the partial fitness components (Table 1). When non-linear selection gradients were analyzed, only the quadratic term flower number substantially improved the adjustment (R^2) of linear models, but usually with lower coefficients than its linear form (results not shown).

Trade-offs and conflicting selection on phenological traits

Phenological traits were selected in opposite ways through some selection episodes depending on the combination of altitude, species and year. Thus, at low and medium altitudes of *S. ciliata* in 2002, selection differentials indicated that flowering onset was positively related to w'_2, but had a final negative effect on plant fitness. That is, the later flowering starts, the greater fruit set is, but the lower plant fitness becomes (Table 1). In *S. ciliata*, selection differentials also showed that flowering duration had a robust positive effect on fitness across most selective scenarios, but on w'_2 (2002, high population) and w'_3

(2003, medium population) the effect was negative. Flower number also had a very strong positive effect on plant fitness, but it had a negative effect on w'_1 (2002, high population), w'_2 (2002, medium and high populations and 2003, high population) and w'_3 (2003, medium population).

In *Armeria caespitosa,* opposing selection was less frequent. Flowering onset was positively related to w'_3, but had a final negative effect on plant fitness (2005, low pasture), while flowering duration was negatively related to w'_2, but had a positive effect on plant fitness (2004, high pasture). Finally, flower number had a general strong positive effect on plant fitness, but a negative effect on w'_2 (2004, high pasture).

Structural equation models

A synthesis of the most parsimonious SEM models is shown in Fig. 2b, c and Table S2. The results of the SEM models were similar to those found with selection differentials and gradients. Of all fitness components initially considered, only the proportion of non-predated flowers developing a mature fruit (w'_2) significantly and consistently affected plant fitness in both *S. ciliata* and *A. caespitosa*. The other fitness components did not significantly affect fitness in spite of presenting significant variability within populations. The most frequent and strongest paths in both species were: flowering duration to flower number, flower number to fitness, and w'_2 to fitness, all of which had positive coefficients (Fig. 2b, c). Other frequent paths found in *S. ciliata* with negative coefficients were flowering duration and flower number to w'_2. The rest of the significant paths were very context-dependent, emerging in less than half of selective scenarios in each species. SEMs also support that flowering onset and duration were under conflicting selection in the same scenarios as those found with selection differentials and gradients (Fig. 3 in appendix, where complete path diagrams and model results of each scenario are shown).

Direct, indirect and total selection emerging from the most parsimonious SEM models is shown in Table 2. Both species showed positive total selection on flowering duration, usually with moderate to large values. In *S. ciliata*, direct selection (direct + indirect effects) on flowering onset was always non-significant and very weak. However, indirect and especially total selection had high negative values in 2002, but it is worthy to note that these cannot be statistically tested. In *A. caespitosa*, direct selection on flowering onset was significantly positive in only two scenarios (2004, low pasture and 2004, high rock, mainly by its indirect effects through flower number). However, once again, indirect and total selection resulted in negative values in most scenarios, especially at low populations in 2005.

Discussion

The understanding of phenotypic selection on flowering phenology is essential for evaluating the potential risks of climate warming, although it has scarcely been explored in high-mountain plants (Stinson 2004; Alatalo and Totland 2003; Sandring et al. 2007). To our knowledge, this is the first study integrating two high mountain plants of contrasting flowering timing in a suite of spatial and temporal selective scenarios.

Results obtained across the different altitudes and microhabitats did not provide a clear pattern. Thus, selection responses for earlier flowering were not stronger at the lower places (i.e., with higher temperature and lower precipitation) as we might have expected. This implies that other local factors, in addition to altitude and microhabitat, affect

Table 2 Summary of the most parsimonious SEM for (a) *S. ciliata* and (b) *A. caespitosa*

	Correlation	Direct selection (β')	Indirect selection	Total selection (s')
(a) Silene ciliata				
2002/Low				
Onset	**−0.630*****	−0.069	−0.205	−0.274
Duration		**0.325***	0.043	0.368
2002/Medium				
Onset	**−0.406*****	−0.141	−0.035	−0.176
Duration		0.086	0.057	0.143
2002/High				
Onset	**−0.412*****	−0.088	−0.103	−0.191
Duration		**0.249****	0.036	0.285
2003/Low				
Onset	0.038	0.013	0.008	0.021
Duration		0.223	0.000	0.223
2003/Medium				
Onset	−0.180	−0.003	−0.042	−0.045
Duration		0.232	0.001	0.233
2003/High				
Onset	0.031	0.068	0.005	0.073
Duration		**0.167***	0.002	0.169
(b) Armeria caespitosa				
2004/Low/Rock				
Onset	**−0.758****	−0.020	−0.168	−0.188
Duration		0.221	0.015	0.236
2004/Low/Pasture				
Onset	**−0.665*****	**0.284****	−0.319	−0.035
Duration		**0.479*****	−0.189	0.290
2004/High/Rock				
Onset	**−0.794*****	**0.586****	−0.470	0.116
Duration		**0.592****	−0.465	0.127
2004/High/Pasture				
Onset	0.046	−0.106	0.014	−0.092
Duration		**0.306*****	−0.005	0.301
2005/Low/Rock				
Onset	**−0.676*****	0.177	−0.417	−0.240
Duration		**0.617*****	−0.120	0.497
2005/Low/Pasture				
Onset	**−0.498*****	−0.237	−0.045	−0.282
Duration		0.090	0.118	0.208
2005/High/Rock				
Onset	−0.052	−0.063	−0.017	−0.080
Duration		**0.323*****	0.003	0.326
2005/High/Pasture				
Onset	−0.269	0.172	−0.120	0.052

Table 2 continued

	Correlation	Direct selection (β')	Indirect selection	Total selection (s')
Duration		**0.446*** **	−0.046	0.400

For simplicity we only show the direct, indirect and total selection exerted by flowering onset and duration on the plant fitness. First column is the correlation between flowering onset and duration, used to calculate the indirect selection through correlational effects (see text)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$. No significance test exists for indirect and total selection because they are obtained by combining correlations and direct selection

reproductive success (Giménez-Benavides et al. 2007a, García-Camacho and Escudero 2009). On the other hand, the effects of temporal variation seemed to predominate over those of spatial variation. The pattern observed in the climatically average years (2002 and 2004) adjusted well to what is expected to happen in high-mountain environments. In general terms, there was a selection towards earlier onset of flowering in both species due to correlational selection through flowering duration (Tables 1 and 2). Nevertheless, the limiting condition brought about by summer drought that we had hypothesized was clearly seen in the late flowering species in 2003, an extreme year with high temperatures and low precipitation (Schär et al. 2004). In this year, snowmelt date occurred approximately 22 days earlier than in 2002, an average year, and flowering onset was also early (Table S1). At the same time, flowering duration was notably lower at all altitudes due to the harsh summer drought. Consequently, earlier flowering onset was not followed by longer flowering duration (Table 2) and early flowering was not selected for plant fitness (Table 1). Thus, early-flowering plants could not take advantage of prolonged flowering in this year (Giménez-Benavides et al. 2007a). In a similar way, a lack of correlation was also found at the high-altitude sites in *A. caespitosa* in 2005, when high temperature and low precipitation patterns were reproduced. In contrast to the favourable outlook for alpine plants (Molau 1993; Alatalo and Totland 2003; Molau et al. 2005), these results support the idea that some plants of Mediterranean high mountains may be highly vulnerable to extreme episodes related to climate warming.

The response of our two model species was more similar between them than expected. We hypothesized that early-flowering species would experience lower selective pressure towards earlier flowering onset because flowering too early might increase pollen limitation due to lower insect activity (Kudo 1991, 1993; Molau 1993; Kudo and Hirao 2006), and because drought would not be a strong selective pressure at the beginning of the growing season, even under Mediterranean conditions. However, our pollen-risk plant did not show evidence of pollen limitation at the beginning of the flowering season (expressed by direct positive effects of flowering onset on fruit set), nor evidence of frost damage during our study period (*personal observation*). Thus, if our observations were representative of the general pattern experienced by this species over the years, *A. caespitosa* could benefit from longer growing seasons in a climate warming context. On the other hand *S. ciliata*, the late-flowering species, also experienced selective pressures towards early-flowering phenotypes. However, its photoperiodic control on flowering time might preclude the use of the most favourable part of the summer for reproduction in the forthcoming warming conditions. Consequently, the different phylogenetic background of the two species affects the ability of the species to respond to these selective episodes.

Our findings also showed that w'_2 (surviving flowers setting mature fruits), a surrogate of pollinator success, is an effective selective episode in both species and the only fitness component from those initially studied that consistently affected plant fitness. It is well known that pollinators may drive the evolution of floral display and flowering phenology (Elzinga et al. 2007; Sandring and Ågren 2009). As expected, w'_2 had a positive, direct effect on plant fitness in all scenarios. Nevertheless, determining how phenological traits are selected through w'_2 is more complex and conflicting. In some scenarios, especially in *S. ciliata*, selection through w'_2 favoured late flowering onset, short flowering duration and the production of few flowers. This suggests that pollen limitation was greater early in the flowering season, whereas low fruitset in plants with many flowers may be due to resource limitation and/or greater fruit abortion due to geitonogamous crossings (Albert et al. 2008). On the other hand, flowering onset, duration and flower number also exerted directional selection on plant fitness, sometimes leading to a suite of opposing selective forces (i.e. plants with early flowering onset, long duration and production of more flowers sometimes had lower w'_2, but in absolute terms produced more fruits). In *A. caespitosa*, however, the constant positive effect of w'_2 on plant fitness was almost independent of phenology and floral display (except in one of eight scenarios), suggesting that pollinator activity does not play a major role in determining flowering phenology in this plant.

Predator-mediated selection may also operate on flowering phenology evolution (Pilson 2000; Elzinga et al. 2007; Ehrlén and Münzbergová 2009; Sandring and Ågren 2009). As biotic interactions and selective forces may differ between altitudes (Gómez and Zamora 2000; Totland 2001; Sandring et al. 2007; Gómez et al. 2009), we expected differences in this fitness component in our studied populations. However, our results did not reveal significant predator-mediated selection on flowering phenology or floral display in either species. In *S. ciliata* 14% of plants were attacked by florivores and 8% by fruit predators, while in *A. caespitosa* 19% of plants were attacked by weevils (mean values across populations and years). However, predation events were fairly evenly distributed throughout the growing season instead of being concentrated in early or late periods, precluding the selection of early or late-flowering individuals.

Our analysis shows an important correlational component of indirect selection between flowering onset and duration that markedly influenced net effects on plant fitness. This correlation was negative and very consistent across species and was responsible for the strength and direction of indirect and total selection as shown in our SEM models (Table 1). In many scenarios, while direct selection (β') indicated a weak phenotypic selection on flowering onset, total selection (s') became negative for this trait after incorporating correlational effects (Table 2). This means that strong phenotypic selection towards early flowering onset might be explained by indirect selection processes. Our SEM diagrams graphically show that constant, moderate selection occurs through a preferential pathway: the negative effect of flowering onset on duration, the positive effect of duration on flower number and, finally, the positive effect of flower number on plant fitness (Fig. 2b, c). As Elzinga et al. (2007) pointed out, the common expectation of stabilizing selection on flowering phenology assumes that, although individuals vary in their first flowering date, they do not vary in their flowering duration. However, when the date of flowering onset is negatively correlated to flowering duration, correlated directional selection takes place.

Our observational approach could have detected spatial variation in selective pressures along the altitudinal gradient and microhabitats considered, indicating local variation in how plants adapt to environmental conditions that vary in space and time. Although phenological traits affected fitness in different ways among sites and years in these two species (see also Giménez-Benavides et al. 2007a; García-Camacho and Escudero 2009), our results suggest that this variation is not very relevant in evolutionary terms. In general, the earlier flowering started, the greater fitness was in both species, and in those scenarios where this pattern was not present, total selection on flowering onset was negligible (Tables 1, S3). Thus, selection of phenological traits was not fluctuating but directional in both species.

In conclusion, our results suggest that flowering onset and duration in these high-mountain Mediterranean species are under selection through an indirect path affecting flower number. When flowering starts earlier, flowering duration is longer and flower number is higher, resulting in a greater number of fruits. Thus, shifts in phenology seem to confer advantages to our model species. Phenotypic selection towards early flowering is consistent in both the early- and late-flowering species studied, but varies in intensity and is context-dependent. We did not detect conflicting selection because w'_2 (surviving flowers setting mature fruits) was the only fitness components initially considered that consistently affected fitness. Future studies should assess whether the selective advantage of early-flowering individuals due to the significant correlation with flowering duration is strong enough to cause the predominance of early-flowering phenotypes in nature. Furthermore, our hypothesis of phenotypic selection on flowering timing should be tested in other high mountain Mediterranean species to explore the evolutionary potential of plants to overcome climate warming.

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