Flowering phenology and reproductive fitness along a mountain slope: maladaptive responses to transplantation to a warmer climate in *Campanula thyrsoides*

J. F. Scheepens · J. Stöcklin

Abstract In many biomes, global warming has resulted in advanced and longer growing seasons, which has often led to earlier flowering in plant taxa. Elevational gradients are ideal to study the effects of global warming as they allow transplantation of plants from their original cooler higher elevations down to elevations with a prospective climate. We transplanted plants from ten populations of the European alpine monocarpic herb species *Campanula thyrsoides* L. to three sites along a steep mountain slope (600, 1,235 and 1,850 m above sea level) in the Swiss Alps and asked whether reproductive phenology adjusts plastically to elevation and if these responses were adaptive, i.e. increased the fitness of plants. We further assessed current genetic differentiation in phenotypic traits and whether any such origin effects were due to adaptation to climatic conditions of origin. Our results showed that transplantation to lower elevations caused strong shifts in phenology, with plants starting growth and flowering earlier than plants placed at higher elevations. However, compared to flower production at high elevation, number of flowers per plant decreased 21 % at mid- and 61 % at low elevation. The shift in phenology thus came with a high cost in fitness, and we suggest that phenology is maladaptive when *C. thyrsoides* faces temperature conditions deviating from its natural amplitude. We conclude that the frequently reported phenological shift in plant species as a response to global warming may include heavy fitness costs that may hamper species survival.

Keywords Common garden · Genetic effects · Local adaptation · Phenotypic plasticity · Swiss Alps

Introduction

In temperate regions, global warming has resulted in an advancement of springtime, leading to earlier flowering in various plant taxa (Abu-Asab et al. 2001; Fitter and Fitter 2002), and a longer period of warm temperatures in summer, resulting in longer growing seasons (Walther et al. 2002; Peñuelas et al. 2002; Cleland et al. 2007). Timing of flowering is important because premature or late flowering can affect seed production and thereby plant fitness (Schemske 1977; Rathcke and Lacey 1985; Cleland et al. 2007), for instance due to pollinator limitation (Schemske 1977; O'Neil 1999), due to smaller nutritive status early in the season (Schmitt 1983; Widén 1991) or due to failing to set seed before the onset of drought, cold temperatures or snowfall late in the season (Giménez-Benavides et al. 2007).

Climate change may impact species persistence in their current distribution, because fine tuning of flowering phenology is tightly linked with temperature patterns during the growing season (Schemske et al. 1978; Rathcke and Lacey 1985; Fitter and Fitter 2002; Quinn and Wetherington 2002). Besides temperature, photoperiod and snowmelt are other important environmental signals for alpine plant species to optimise flowering time during the growing season (Rathcke and Lacey 1985; Huelber et al. 2006). Interactions among these environmental signals may complicate an easy adjustment to climate change. Research

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suggests that some plant species experiencing an advanced and longer growing season may have less time after snowmelt to acquire resources before environmental cues cause the onset of flowering (Post et al. 2008), leading to lower fitness (Burgess et al. 2007; Haggerty and Galloway 2011). Additionally, plants may accelerate flowering in response to rapidly increasing temperatures under future climate conditions resulting in fewer flowers (Sherry et al. 2007; Haggerty and Galloway 2011). Climate change may also lead to a mismatch between anthesis and pollinator availability, especially when phenology is accelerated, and may therefore result in decreased reproductive output (Schemske et al. 1978; Elzinga et al. 2007).

Phenotypic plasticity allows for faster adjustments to changing environmental conditions than the much slower local adaptation through natural selection (Bradshaw 1965; Schlichting 1986; Thompson 1991; Sultan 2000; Hoffmann and Sgrò 2011). Therefore, plasticity is thought to be favoured in temporally and spatially heterogeneous environments (Van Kleunen and Fischer 2005; Banta et al. 2007; Stöcklin et al. 2009). For instance, it may be beneficial for plants to plastically adjust their flowering phenology to temperature variability under highly variable springtime temperature and weather conditions (Quinn and Wetherington 2002). Plasticity in phenological timing may also be beneficial for survival under future stress of climate change, with more plastic species having an advantage over species with a genetically fixed phenology (Sultan 2000; Ghalambor et al. 2007). However, plastic responses to warmer and longer growing seasons may also turn out maladaptive when they are accompanied with decreases in fitness (Rathcke and Lacey 1985; Alpert and Simms 2002; Ghalambor et al. 2007). Maladaptive responses may particularly result when future environmental conditions lie outside the range of naturally experienced variation (Ghalambor et al. 2007). Global warming is predicted to continue in the near future, so it is important to quantitatively assess the adaptive potential of plant species to increased temperatures.

Elevation gradients are ideal for studying the effects of global warming because they allow transplantation of plants from their original higher elevation to lower elevation, thereby simulating a warmer climate while holding other factors (e.g. day length and regional weather patterns) relatively constant (Haggerty and Galloway 2011). Similar to global warming, the most important change to occur with decreasing elevation is an increase in the average temperature, and the growing season to start earlier and to last longer (Ozenda 1988; Körner 2003). Although temperature is the main factor affecting plant growth (Dahlgren et al. 2007), especially across elevational common gardens, other factors such as soil characteristics, irradiance and pollinator abundance that also vary with elevation can confound such studies (Körner 2003; Dahlgren et al.

2007). Nevertheless, field experiments are preferable to studies in the greenhouse or climate chamber where controlled temperature and precipitation conditions may deviate strongly from natural weather patterns (relevant when, for instance, snowmelt plays a role in phenology).

After transplanting plants to common gardens at several elevations along a slope, phenotypic traits, including phenology, may respond plastically to the elevation treatment and may also reveal genetic effects restricting change (Scheepens et al. 2010; Haggerty and Galloway 2011). Thus, an elevational treatment allows separating genetic and plastic responses to that treatment (Scheepens et al. 2010; Vitasse et al. 2010; Ebeling et al. 2011) yielding insight in the ability or inability of traits to adapt to rapid environmental change.

We transplanted *Campanula thyrsoides* L. from ten populations in the Swiss Alps to three sites along a steep mountain slope [600, 1,235 and 1,850 m above sea level (a.s.l.)] and asked whether plastic adjustments in phenology and reproductive traits to the elevation treatment were constrained by the current genetic constitution of the plants (i.e. origin effects). We further investigated whether any genetic effects in phenotypic traits may be due to adaptation to climatic conditions of origin.

C. thyrsoides is a subalpine/alpine bell flower occurring throughout the European Alps and Jura Mountains. We chose to study *C. thyrsoides* since it is one of the few monocarpic perennial Alpine species and produces an inflorescence with easily countable flowers, which allows for relatively accurate fitness estimates after flowering. The genus *Campanula* is known to have variable requirements for initiation of flowering (Padhye et al. 2005), and it is currently unknown how *C. thyrsoides* responds to photoperiod, temperature or snowmelt.

The objective of our study was to experimentally address the following questions: (1) Is reproductive phenology advanced at lower elevations compared to higher elevations? (2) Is this response adaptive or does it come with a fitness cost? (3) How much of the variation in phenology and reproductive traits is due to plastic responses and how much is due to genetic effects? (4) If genetic effects are present, can they be explained as adaptation to sites of origin?

Materials and methods

Study species and sampled populations

Campanula thyrsoides is a monocarpic perennial bell flower occurring in subalpine to alpine grasslands with calcareous soils or carbonate-rich schist. Individuals growing in their natural habitat flower after 3–15 years' growing as a rosette

Table 1 Locations of sampled *Campanula thyrsoides* populations and the experimental sites

Population	Code	*n*	Northing	Easting	Elevation	Prec[a]	Temp[a]
Sampling sites							
Trient, Les Tseppes	TRI	84	46°02′53.93″N	6°58′47.05″E	2,020	1,672	1.9
Luc du Fully	FUL	72	46°10′09.96″N	7°06′09.51″E	2,100	1,780	1.0
Lac du Moiry	MOI	84	46°08′12.78″N	7°34′02.87″E	2,266	1,827	−0.1
Stockhorn	STO	48	46°41′37.05″N	7°32′16.72″E	1,980	1,700	2.3
Schynige Platte 4	SP4	84	46°39′17.31″N	7°54′16.67″E	1,911	1,716	2.0
Schynige Platte 18	SP18	87	46°39′33.73″N	7°55′14.41″E	1,930	1,716	2.0
Churwalden, Joch	CHJ	84	46°47′51.41″N	9°33′53.65″E	1,890	1,520	0.2
Langwies, Holzbueel	LAH	84	46°49′41.97″N	9°44′00.53″E	1,700	1,095	3.6
Langwies, Listboden	LAL	84	46°51′07.02″N	9°45′32.22″E	2,000	1,326	1.1
Ftan Prui	FTA	83	46°48′32.68″N	10°13′20.37″E	2,101	1,383	−1.1
Experimental sites							
Low site		250	46°52′18.34″N	9°31′05.45″E	600	484	19.2
Intermediate site		252	46°53′11.29″N	9°29′42.76″E	1,235	439	16.0
High site		253	46°52′48.69″N	9°30′36.10″E	1,850	698	12.6

n Sample size of individuals in the common garden, *Prec* precipitation, *Temp* mean temperature

[a] For sampling sites: mean annual precipitation and temperature, based on monthly averages; WorldClim, Hijmans et al. 2005. For experimental sites: total precipitation and mean temperature, based on weather station data from 3 June to 28 Oct 2009 (see main text)

(Kuss et al. 2007). Flowering is dependent on rosette size (Kuss et al. 2008), occurring only in plants that are sufficiently large. Under favourable conditions (e.g. in cultivation), the plant behaves as a biennial. The inflorescence bears on average (mean ± SE) $50 ± 9$ bright yellow flowers (Scheepens et al. 2011) each with on average $147 ± 91$ seeds (Kuss et al. 2007). Seeds lack adaptations for dispersal and fall close to the mother plant, making pollen dispersal, predominantly by bumblebees, the most important factor for gene flow (Scheepens et al. 2012).

The species is distributed across the European Alps and the Jura Mountains with additional satellite populations in the Dalmatian Mountains, covering an elevation gradient from 217 to 2,900 m a.s.l. (Kuss et al. 2007). Two subspecies have been recognized (Scheepens et al. 2011). The subspecies *carniolica* occurs at comparatively lower elevations (mean ± SD of ten populations: $763 ± 389$ m a.s.l.; Kuss et al. 2011) mainly in the Slovenian part of the Alps, whereas the main subspecies *thyrsoides*, to which we restricted our study, occurs throughout the rest of the Alps, with most populations residing at 1,600–2,200 m a.s.l., and in the Jura Mountains at 1,300–1,700 m a.s.l. (Kuss et al. 2007, 2011). Seed families were sampled from ten populations ranging from western to eastern Switzerland and covering an elevational range of 566 m (Table 1).

Common garden experiment

In the autumn of 2007, we germinated seeds collected from each population on wet filter paper in Petri dishes. We then transplanted seedlings to pots with a diameter of 5 cm and a height of 6 cm (multi-trays) filled with nutrient-poor soil (Anzuchterde; Ökohum, Herrenhof, Switzerland). Greenhouse conditions buffered natural temperature and irradiation extremes and water was added manually. We grew 12 offspring per seed family and 7 families per population, i.e. 84 individuals per population, except for populations FUL and STO that had 6 and 4 seed families, respectively (Table 1). After 10–18 weeks, we transplanted the young plants to bigger pots (10 × 10 × 10 cm). We placed the plants outside the greenhouse to acclimate before final transplantation to the three elevational sites.

Three fenced common gardens were located in pastures on the Calanda mountain, Switzerland, at 600, 1,235 and 1,850 m a.s.l. along a single southeast-facing mountain slope with distances not exceeding 2,500 m (based on longitude and latitude). *C. thyrsoides* does not naturally occur in the selected pastures. We measured the air temperature and precipitation at the three sites twice daily (at 10:00 and 22:00 hours) from 3 June to 28 Oct 2009 using WS-2300 Matrix weather stations (LaCrosse Technology, La Crosse, WI, USA). Since the sampled populations of *C. thyrsoides* originated from elevations of (mean ± SD) $1,990 ± 143$ m a.s.l., the upper garden best reflected their natural elevation, whereas the mid- and low-elevation gardens can be considered as global warming treatments of increasing severity (1,235 m a.s.l.: +3.4 °C; 600 m a.s.l.: +6.6 °C; Table 1).

On 24 June 2008 ($T_{June2008}$), four plants per seed family were randomly transplanted to each garden into the local soil, which had its natural plant cover removed, was

subsequently spaded 50 cm deep, and was topped with a layer of sterilised soil to suppress weeds. *C. thyrsoides'* long tap root hampers the use of pots or plots with a layer of standardised soil. On the slopes, we constructed horizontal plant beds with wooden planks to prevent the soil from being washed or pushed away by precipitation. The low, intermediate and high sites contained 266, 264 and 264 plants, respectively. The number of leaves and rosette diameter were recorded upon transplanting and plants were grown for two growing seasons, 2008 and 2009. On 2 Sept 2008 ($T_{Sept2008}$), we again recorded the rosette diameter as well as the phenological stage. We recognised the following four phenological stages: (1) *dead*, when no photosynthetically active tissue is present and the plant has not flowered; (2) *rosette*, when the plant is alive and has not yet started flowering; (3) *flowering*, when the plant shows any signs of growing one or more inflorescences; and (4) *ripening*, when all flowers on all inflorescences had passed through their receptive phase.

In the second growing season, on 19–25 May 2009 ($T_{May2009}$), we assessed the phenological stage and measured the rosette diameter as well as the following reproductive traits: number of inflorescences, maximum inflorescence height and number of flowers. Five weeks later, on 21–22 June 2009 ($T_{June2009}$), we again assessed the phenological stage and measured the same reproductive traits. Finally, at the end of the second growing season, on 9–13 Sept 2009 ($T_{Sept2009}$), we last measured the reproductive traits. During each measurement in 2009, we harvested the above-ground biomass of the plants that had flowered and had ripened their seeds. This biomass was weighed after drying for 72 h at 60 °C in an oven. We considered biomass a reproductive trait because the bulk of the biomass consisted of inflorescences with leaves contributing very little. For observations of reproductive traits, we used data collected at the last available measurement for further analysis.

Data analysis

Phenology

G tests were performed to assess the effect of elevation treatment (the three common gardens) on phenological stage (based on Sokal and Rohlf 1995, with R code from Pete Hurd, University of Alberta, 2001) at three time points: $T_{Sept2008}$, $T_{May2009}$ and $T_{June2009}$. We also analysed the effect of populations on phenology at these time points to investigate whether populations were genetically differentiated in phenology.

Phenotypic trait variability and fitness

Generalised linear mixed-effects models (Crawley 2007) were used to analyse the effects of elevation treatment,

population origin and seed family on measured vegetative and reproductive traits from the common garden experiment. Rosette diameter at the start of the experiment was included as a covariate to account for differences which may have their origin in various germination dates or maternal effects. However, when analysing rosette diameter at the start of the experiment as dependent variable, we used number of leaves at the start of the experiment as covariate. Elevation treatment assessed the effect of transplantation to different elevations on the measured traits and was included as a fixed factor. Genetic effects of populations as well as seed families within populations were assessed by treating these factors as random effects. The interactions of elevation treatment with population and seed family were interpreted as genetic differences in plasticity and were treated as random effects. The linear mixed-model analyses were performed using type I sum of squares, and residuals were checked visually to see if the assumptions of normality were met. We further checked our data for homogeneity of variance using Levene's test. Data on rosette diameter at $T_{Sept2008}$, above-ground biomass and number of flowers were square-root transformed to improve normality and homogeneity of variance. Number of inflorescences was analysed using a Poisson distribution with log link function, whereas the other traits were assessed using a normal distribution with identity link function. Data dispersion of the Poisson model was checked and data was found to be underdispersed, indicating less variation in the data than predicted, which was ignored (Zuur et al. 2009). χ^2 values and significances of model factors were derived from likelihood ratio tests of model comparisons using maximum likelihood (Crawley 2007), starting stepwise deletion with interactions.

The number of flowers was used as a proxy for the number of seeds produced, which is a good estimate of maternal fitness in monocarpic perennials (Metcalf et al. 2003). An earlier survey including individuals and populations from the entire range of the species showed that the number of flowers on an inflorescence was positively related to fitness measured in terms of the number of well-developed seeds per flower capsule (Pearson's $r = 0.39$, $P < 0.01$; H.H. Ægisdóttir, unpublished results). However, traits such as survival and above-ground biomass are obviously also linked to fitness.

We estimated variance components to assess the importance of plastic versus genetic effects by performing similar mixed-effects models with all factors treated as random and using restricted maximum likelihood (Crawley 2007). The variance explained by the elevation treatment represents the phenotypic plastic response, variance explained by populations and seed families represents genetic differences, and variance explained by the interactions of elevation treatment with populations and seed

families represents genetic differences in phenotypic plasticity among populations or seed families. The latter is a measure of the heritable component of phenotypic plasticity (Schlichting 1986; Thompson 1991).

We also analysed post-transplantation mortality and mortality over winter 2008–2009 using a similar mixed-effects model as above but with a binomial distribution with logit link function to analyse the binary data, which we checked for overdispersion.

Adaptation to conditions of origin

As a first step to investigate adaptation to conditions at the sites of origin, we analysed population means of phenotypic traits for the three elevational sites ($n = 30$) as dependent variable and elevation treatment as explanatory variable using linear models. Population means used in these models were based on seed family means. We then used the average residuals per population of these models for Pearson's correlations between traits and environmental conditions at the sites of origin ($n = 10$). Environmental variables included elevation of population origin, mean annual temperature and annual precipitation. Temperature and precipitation data were obtained from the WorldClim database for each population location (Hijmans et al. 2005). Out of four WorldClim grid points surrounding a population location, we chose the one with the least elevational difference to the population. Temperature was then adjusted by adding or subtracting 0.0055 °C per metre elevational difference with the population. Precipitation was taken from the same grid point.

To investigate whether the width of phenotypic plasticity could be explained as adaptation to local conditions, we correlated the coefficient of variation for each trait measured across the three gardens with elevation, annual precipitation and annual mean temperature of origin.

All analyses were performed in R (R Development Core Team 2009).

Results

Mean temperature between 3 June and 28 Oct 2009 was 19.2 °C at the lowest site, 16.0 °C at the intermediate site and 12.6 °C at the highest site. Total precipitation over this period was 484 mm at the lowest site, 439 mm at the intermediate site and 698 mm at the highest site (Table 1).

Phenological changes due to transplantation to lower elevations

The majority of plants flowered during the second growing season, in 2009. G tests showed that the distribution of

phenological stages was significantly different among the elevational gardens at T_{May2009} and T_{June2009} (Table 2a), whereas this did not differ among elevations at T_{Sept2008}. The percentage of flowering individuals decreased sharply with increasing elevation in the second year at T_{May2009} (i.e. 46 % at low elevation, 26 % at mid-elevation, 0 % at high elevation; Fig. 1a), mortality at T_{May2009} and T_{June2009} was lowest at mid-elevation, and at T_{June2009} a higher percentage of plants from the intermediate site were still in their rosette phase compared to plants from the low and high sites (Fig. 1a). Additionally, seeds started ripening later with increasing elevation (J.F. Scheepens, personal observation). Thus, transplantation of plants to lower elevation (i.e. warmer climate) caused strong shifts in phenology, with plants starting to flower earlier, and ending their reproductive cycle earlier than they did at higher elevation.

We also found effects of population origin on phenology at all three census dates (Fig. 1b; Table 2b), indicating genetic differences in phenology among populations. G tests on data collected from each elevation separately also showed that populations differed in phenology ($P < 0.05$) in six out of nine tests (elevation × census date), but beta regressions of percentage flowering could not explain phenology of populations with elevation of origin at the three sites (low elevation: $z = -0.62$, $P = 0.5$; mid: $z = 0.15$, $P = 0.8$; high: no flowering plants).

Plasticity and genetic differentiation in phenotypic traits

Most plants flowered in the second growing season (2009; Fig. 1a) and the linear mixed-effects models showed that elevation treatment had a strong effect on rosette diameter and reproductive traits (Table 3). The only exception was rosette diameter at T_{June2008}, since this trait was measured at the day of planting. Rosette diameter at T_{Sept2008} was higher at the low site compared to the intermediate and high site, and rosette

Table 2 Results of G tests for independence between elevation treatment and (a) phenological stage and (b) population in *Campanula thyrsoides*

	df	G	P
(a) Phenological stage versus elevation treatment			
T_{Sept2008}	2	3.1	0.21
T_{May2009}	4	268.4	<0.0001
T_{June2009}	4	65.8	<0.0001
(b) Population versus elevation treatment			
T_{Sept2008}	9	65.2	<0.0001
T_{May2009}	18	47.5	0.0002
T_{June2009}	18	46.1	0.0003

T indicates the time of phenological assessment
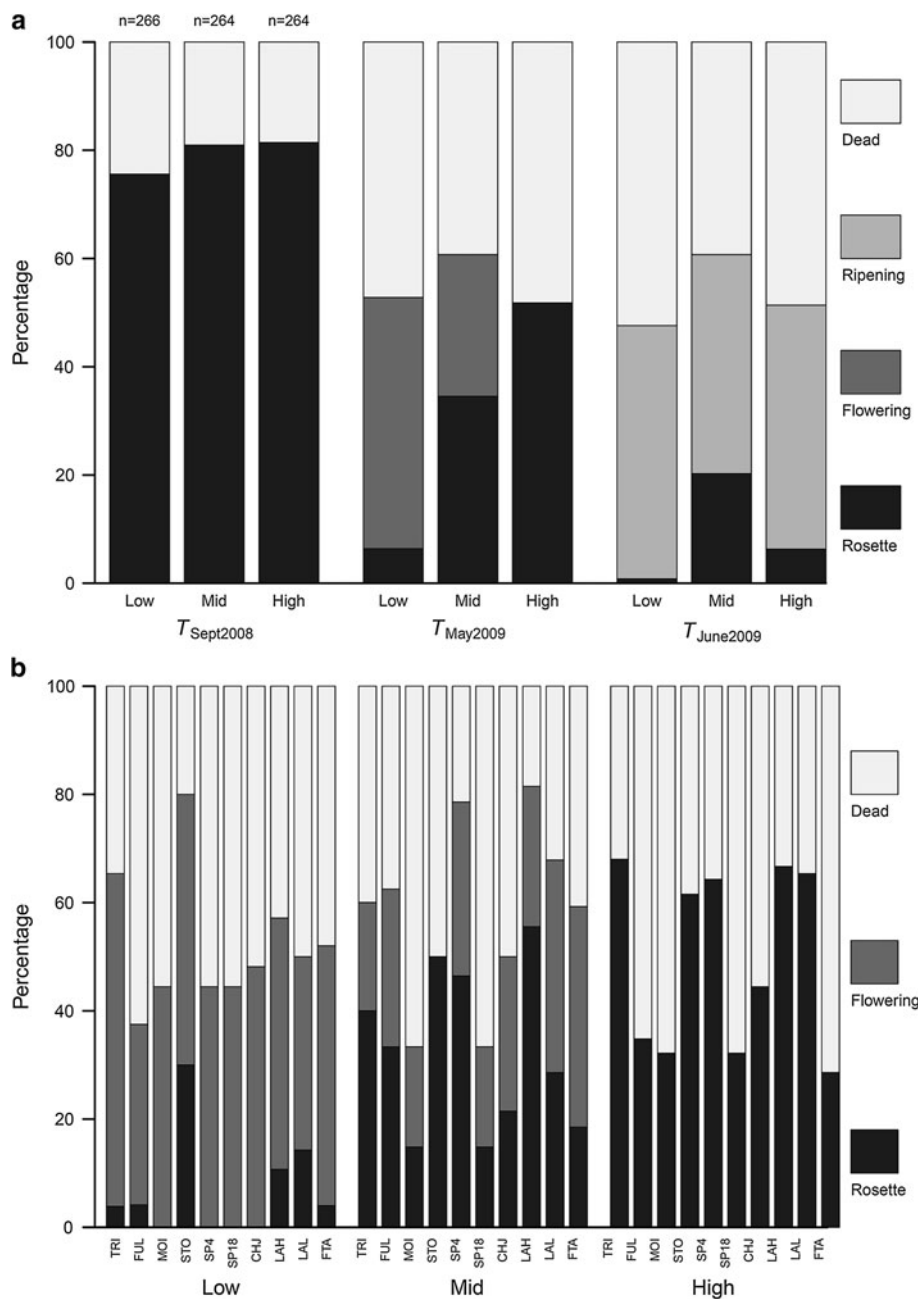
diameter at $T_{May2009}$ increased at the low and intermediate sites but remained unchanged at the high site (Fig. 2). This probably reflected the advanced growing season and the correspondingly advanced phenology after transplantation to lower elevations, or faster growth at lower elevations.

Reproductive traits generally decreased after transplantation to lower elevations (Fig. 3): above-ground biomass decreased at low and intermediate elevations, as did the number of inflorescences at the low site, compared to that observed at the high site. Maximum height and number of flowers also decreased strongly with transplantation to lower elevations.

The number of flowers per plant decreased significantly when plants were transplanted to lower elevation sites (21 and 61 % reductions when transplanted to mid- and lowest elevations, respectively; Fig. 3d; Table 3). This corresponds to a fitness loss of 17 flowers per plant (9 % of performance at highest site) per K temperature increase (c. 182 m elevational difference).

The linear mixed-effects models (Table 3) further showed that the covariate, i.e. rosette diameter at the start of the experiment, was significant for all traits except for number of inflorescences. This generally indicated that plants with larger rosette size at the start of the experiment

Table 3 Results of mixed-effects models testing the effects of transplantation to different common gardens (i.e. elevation treatment), population of origin, seed family and interactions of elevation treatment with populations and seed families on measured phenotypic traits of *Campanula thyrsoides*

	Rosette diameter $T_{June2008}$				Rosette diameter $T_{Sep2008}$				Rosette diameter $T_{May2009}$				Above-ground biomass			
	df	χ²	P	% V	df	χ²	P	% V	df	χ²	P	% V	df	χ²	P	% V
Rosette diameter $T_{June2008}$[a]	1	51.6	**<0.0001**	33.9	1	12.9	**<0.001**	0.0	1	23.9	**<0.0001**	0.0	1	36.5	**<0.0001**	0.1
Elevation treatment	2	1.2	0.55	0.0	2	19.0	**<0.0001**	4.3	2	174.9	**<0.0001**	47.1	2	17.0	**<0.001**	6.4
Population	1	172.5	**<0.0001**	14.4	1	173.0	**<0.0001**	30.2	1	18.4	**<0.0001**	6.3	1	18.2	**<0.0001**	14.8
Seed family (population)	1	26.5	**<0.0001**	6.0	1	3.3	0.07	1.3	1	3.3	0.07	1.2	1	1.1	0.30	3.4
Elevation treatment × Population	1	0.0	0.99	0.38	1	0.1	0.81	0.0	1	1.1	0.28	0.6	1	0.0	1.00	0.0
Elevation treatment × Seed family (population)	1	0.0	1.00	0.0	1	2.5	0.12	5.4	1	8.5	**<0.01**	11.2	1	0.0	1.00	0.0
Residuals	761			45.3	591			58.9	345			33.5	304			75.3

	Maximum height				Number of inflorescences				Number of flowers			
	df	χ²	P	% V	df	χ²	P	% V	df	χ²	P	% V
Rosette diameter $T_{June2008}$	1	32.2	**<0.0001**	3.2	1	2.5	0.12	0.0	1	22.4	**<0.0001**	1.4
Elevation treatment	2	171.3	**<0.0001**	47.3	2	7.8	**0.02**	0.7	2	113.9	**<0.0001**	37.8
Population	1	22.7	**<0.0001**	8.0	1	17.8	**<0.0001**	4.4	1	21.6	**<0.0001**	4.3
Seed family (population)	1	2.1	0.14	2.7	1	0.0	1.00	0.0	1	0.6	0.46	2.2
Elevation treatment × Population	1	0.1	0.75	0.2	1	0.0	1.00	0.0	1	12.0	**<0.001**	7.6
Elevation treatment × Seed family (population)	1	0.0	1.00	0.0	–	–	–	–	1	0.5	0.48	2.7
Residuals	327			38.6	328			94.9	313			44.1

Significant values shown in bold

[a] For the analysis of rosette diameter $T_{June2008}$ as dependent variable, we used number of leaves as covariate
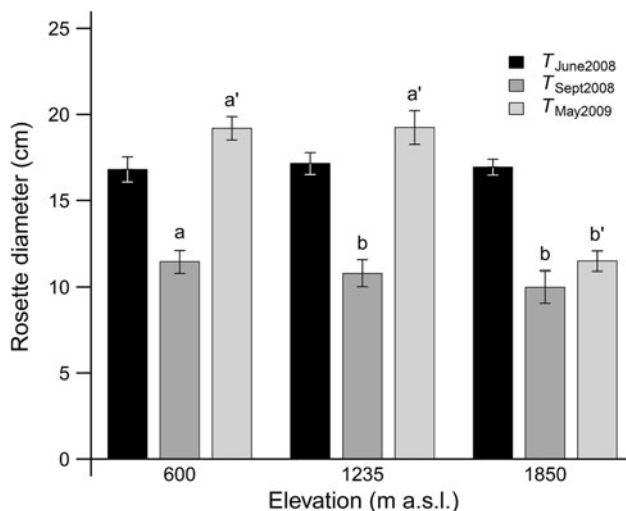
Fig. 2 Responses of rosette diameter in *Campanula thyrsoides* plants measured at three points in time ($T_{June2008}$, $T_{Sept2008}$, $T_{June2009}$) and grown in common gardens at three elevations. Means and standard errors are based on population means, which in turn are based on seed family means. *Letters* indicate significant differences among the common gardens at different elevations for given time points based on Tukey HSD tests

had higher reproductive trait values (Pearson's $r = 0.23$, $P < 0.0001$).

All traits showed origin effects, indicating genetic differences among populations. Seed families within populations responded differently for the dependent variable rosette diameter $T_{June2008}$, indicating genetic differentiation among seed families within populations in rosette diameter at the start of the experiment.

Number of flowers showed an interaction between the elevation treatment and population, probably because populations LAH and FTA (Table 1) showed a different pattern across elevational sites for this trait than did the other populations. Rosette diameter at $T_{May2009}$ showed an interaction between the elevation treatment and seed family, indicating that seed families responded differently at different elevations.

Results from the variance component analysis showed that variance explained by plastic responses (elevation treatment) was large compared to genetic effects (populations and seed families) for rosette diameter at $T_{May2009}$ (47.1 %), maximum height (47.3 %) and number of flowers (32.5 %; Table 3). Conversely, genetic differentiation

Fig. 3 Responses of fitness-related phenotypic traits in *Campanula thyrsoides* plants to transplantation to three common gardens. Means and standard errors are based on population means, which in turn are based on seed family means. *Letters* indicate significant differences among the common gardens at different elevations based on Tukey HSD tests
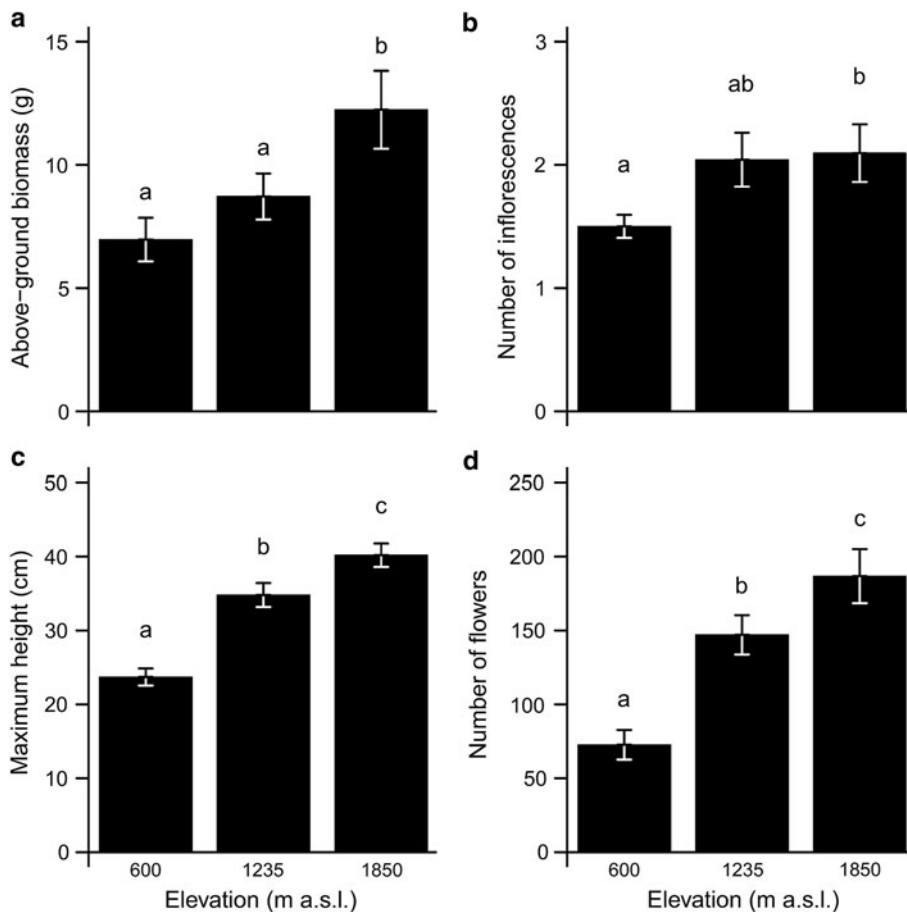
among populations and seed families and through interactions with the elevation treatment was larger than plastic responses for rosette diameter at $T_{June2008}$ (20.8 %) and at $T_{Sept2008}$ (37.9 %), biomass (18.4 %) and number of inflorescences (4.4 %; Table 3).

Mortality occurring between planting and the subsequent measurements was high (21 %) in our study (Fig. 1a) and might have been due to a transplant shock. The generalised mixed-effects model showed that 16 % of variance in mortality was due to three tested factors (Table 4): rosette diameter, population of origin, and seed family, leaving most variation unexplained. No variable could explain variance in mortality over the winter.

Adaptation to conditions of origin

Adaptation was suggested by model residuals of biomass for each population ($n = 10$) correlating negatively with increasing elevation of origin ($r = -0.84$, $P < 0.01$; Fig. 4a) and positively with mean temperature ($r = 0.77$, $P < 0.01$; Fig. 4b). Thus, plants coming from higher, and therefore colder, sites had lower biomass. Average maximum plant height also correlated positively with mean temperature ($r = 0.82$, $P < 0.01$; Fig. 4c), meaning that plants originating from colder locations were shorter than plants originating from lower elevations. No other trait-environment correlations were found, even though number of flowers correlated positively with biomass ($r = 0.67$, $P = 0.04$) and maximum height ($r = 0.69$, $P = 0.03$). Since 24 correlations were tested, we suppose that three significant correlations were unlikely to be due to chance effects through multiple testing. Thus, the correlations suggest that populations were genetically adapted to the temperature regime at their respective sites of origin.

When investigating local adaptation in the magnitude of plasticity, none of the correlations between the coefficient of variation in phenotypic traits across the elevational sites and environmental variables at the sites of origin were statistically significant, with the exception of number of flowers. Plasticity in this trait was positively related to annual mean temperature ($n = 10$, $r = 0.66$, $P = 0.04$). However, out of 24 correlations, this single significant result could well be due to chance effects through multiple testing and should therefore be interpreted with caution.

Table 4 Results of generalised mixed-effects models testing the effects of the elevation treatment, population of origin, seed family and interactions of elevation treatment with populations and seed families on mortality of *Campanula thyrsoides* plants in the common gardens (A) between $T_{June2008}$ and $T_{Sept2008}$ and (B) between $T_{Sept2008}$ and $T_{May2009}$

	(A) Mortality $T_{June2008}$—$T_{Sept2008}$				(B) Mortality $T_{Sept2008}$—$T_{May2009}$			
	df	χ^2	P	% V	df	χ^2	P	% V
Rosette diameter $T_{June2008}$	1	9.5	**<0.01**	4.5	1	11.1	**<0.001**	0.1
Elevation treatment	2	3.1	0.29	0.2	2	5.8	0.06	0.8
Population	1	29.1	**<0.0001**	6.1	1	0.5	0.49	0.0
Seed family (population)	1	7.8	**<0.01**	5.5	1	0.5	0.46	1.5
Elevation treatment × population	1	0.0	1.00	0.0	1	1.6	0.20	2.9
Elevation treatment × seed family (population)	1	0.1	0.79	1.9	1	0.0	0.92	1.3
Residuals	747			81.8	592			93.4

Significant values shown in bold
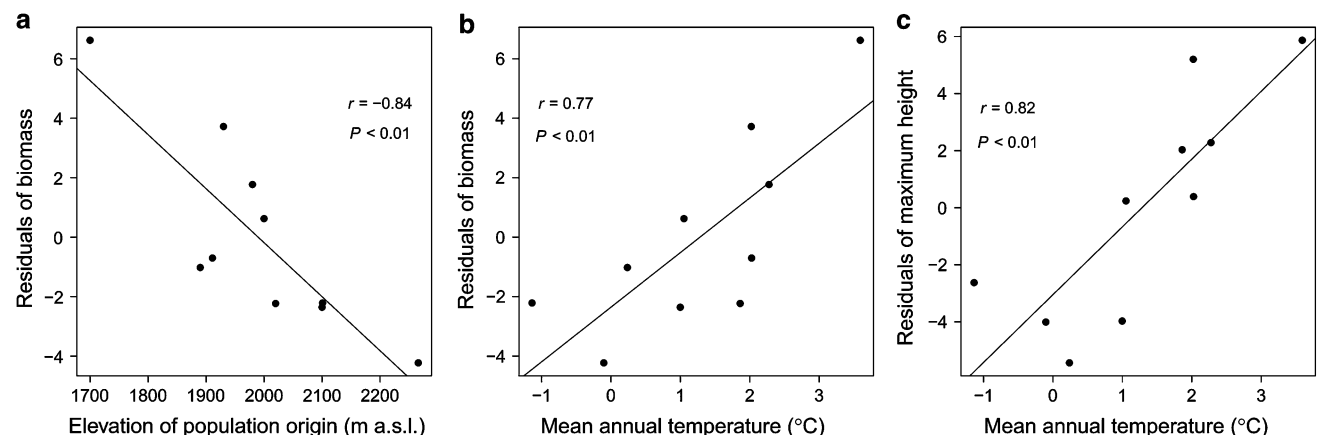
Fig. 4 Pearson's correlations between model residuals of mean trait values of **a**, **b** biomass and **c** maximum height for each population of *Campanula thyrsoides* ($n = 10$) with (**a**) elevation of origin or (**b**, **c**) mean annual temperature

Discussion

Phenology and fitness

Our results showed that transplantation of the monocarpic alpine herb *Campanula thyrsoides* to lower elevations (i.e. warmer climate) caused strong shifts in phenology. Plants started growth earlier, flowered earlier, and finished their reproductive cycle earlier than they did at the highest elevation. Flowering initiation in *C. thyrsoides* is thus shown to not be exclusively determined by photoperiod, but by a large extent to be regulated by temperature. Such an advancement of phenology after transplantation to lower elevations has commonly been found (e.g. Trtikova et al. 2010; Vitasse et al. 2010; but see, e.g., Monty and Mahy 2009).

The shift in phenology came with a high cost in fitness, reflected by the lower number of flowers and inflorescences, and by the decreased maximum height and aboveground biomass at lower elevation sites relative to plants grown at the highest elevation. This indicates that *C. thyrsoides*' plastic response after transplanting to lower elevations was maladaptive.

In alpine environments, where the growing season can be short and highly variable from year to year and seed set must be fulfilled before the end of the season, flowering in many species is strongly regulated by temperature (Bliss 1971; Walker et al. 1995; Huelber et al. 2006; Kudo and Hirao 2006). Such phenological fine-tuning to temperature may also lead to accelerated phenology when air temperature patterns progress fast, regardless of whether this is a passive plastic response of faster plant development (Haggerty and Galloway 2011) or an adaptation to ensure successful seed production during short growing seasons at high elevation. After transplantation to lower elevations (or due to climate change), plants may face having less time for vegetative growth between snowmelt and the environmental cue to start flowering (Post et al. 2008). Alternatively, they may respond with accelerated flowering in response to rapidly increasing temperatures at lower elevations. Both potentially result in fewer flowers in species with determinate flowering (Haggerty and Galloway 2011). We propose that, in our experiment with *C. thyrsoides*, phenology may not only have been advanced but also accelerated at the lower sites. This might have been a response to faster transition from winter to summer conditions compared to a slow transition occurring at the highest site (based on weather data of 2011 provided by Y. Vitasse), leading to earlier termination of flower production on the inflorescences. We suggest that plastic changes in phenology may have caused the observed fitness loss after transplantation to lower elevations. A maladaptive response similar to ours was found in a study with *Campanulastrum americanum* plants transplanted reciprocally

between two contrasting elevations (Haggerty and Galloway 2011). Likewise, Sherry et al. (2007) found reduced reproductive time spans under a warming treatment in four out of nine prairie plants flowering before peak summer, and Post et al. (2008) found comparable results for a forb and two shrub species in the arctic.

We discount the role of water stress to explain decreased performance at lower elevations (Linares et al. 2012), since formation of the determinate inflorescence occurs early in the growing season (Bull-Hereñu and Claßen-Bockhoff 2010; Scheepens et al. 2011). Also, herbivory pressure and pollinator availability, although not affecting our results on number of flowers, did not seem to differ across sites. Soil conditions were not standardised across the sites and mycorrhizal infection rates may also have differed, potentially confounding our explanation that a shifted phenology caused the fitness decline. However, the strong phenotypic differences across the large elevational gradient suggest the role of factors related with elevation, most likely temperature patterns and the associated snowmelt date (Körner 2003; Huelber et al. 2006; Haggerty and Galloway 2011).

As our study of *C. thyrsoides* populations originated from elevations between 1,700 and 2,266 m a.s.l., the highest common garden site was still within this species' natural range, whereas the intermediate and lowest sites were outside the range occupied by most populations of *C. thyrsoides* in the Swiss Alps. The observed plastic response of plants to start flowering earlier in environments with advanced spring temperatures (and to presumably accelerate flowering phenology) under rapid springtime warming is likely to be adaptive within its native range. However, when plants were transplanted to elevations outside this range, this plastic response was no longer adaptive and led to strong fitness losses (Ghalambor et al. 2007). In fact, our results allow the interpretation that local adaptation is one of the causes of the maladaptive response of the plants, since the observed plastic response has evolved in the species' particular alpine environment and, as a genetic trait, is likely adaptive only within, but maladaptive outside, the native range of *C. thyrsoides*.

In addition to the 10 populations from the Swiss Alps, we transplanted 84 plants from each of two populations from the Jura Mountains, originating from 1,340 to 1,440 m a.s.l., to the three elevational sites, with their elevations of origin falling in between the intermediate and highest site. In line with our reasoning above, these plants did not show differences between the intermediate site and the highest site in terms of biomass, maximum plant height, and the number of inflorescences and flowers, but they did show 43 % lower maximum height and 37 % fewer flowers at low elevation than at intermediate elevation (data not shown).

Plasticity and genetic differentiation in phenotypic traits

Results showed strong plastic responses of phenotypic traits and in phenology to elevation, as well as genetic differentiation in phenotypic traits and in phenology among populations and seed families. Moreover, the significant interaction between elevation and population origin for number of flowers indicated that substantial genetic differentiation in phenotypic plasticity was present among populations, which is an indication that plasticity itself can be under selection (Thompson 1991), strengthening its influence on fitness.

Common garden studies applying one or more treatments have usually found both plastic and genetic effects (Scheepens et al. 2010; Vitasse et al. 2010), and the importance of both may vary among traits, populations and species (Lemke et al. 2012). Our variance component analysis showed larger plastic effects than genetic differentiation for rosette diameter in 2009, maximum height and number of flowers, but stronger genetic differentiation than plasticity for rosette diameter at $T_{June2008}$ and $T_{Sept2008}$, above-ground biomass, and number of inflorescences. This indicated that traits differ in the way they adjust to the environment, which may reflect their response to either gradual environmental changes (by genetic adaptation) or strong spatial and temporal heterogeneity (by phenotypic plasticity; Banta et al. 2007).

Adaptation to conditions of origin

Our data showed that populations differed considerably in growth and phenology, that elevation of origin is one of the most likely (indirect) environmental variables to which plants are adapted, and that some phenotypic traits were highly correlated with environmental conditions at origin. These results suggest local adaptation in the sampled populations across the Swiss Alps. The inflorescences of plants from higher elevations of origin, corresponding to colder environments, weighed less and, with decreasing temperatures, inflorescences, also became smaller. Similar patterns in plant growth, root:shoot allocation and reproductive traits have been found in other alpine species (Byars et al. 2007; Gonzalo-Turpin and Hazard 2009; Hautier et al. 2009; Monty and Mahy 2009; Fischer et al. 2011; Scheepens and Stöcklin 2012). Thus, it seems that, with increasing elevation, perennial plants show more conservative investment in vegetative structures and constrained investment in reproduction over their lifetime (Von Arx et al. 2006). However, adaptation to elevation does not show a general pattern, as numerous studies point out (e.g. Stinson 2004; Byars and Hoffmann 2009; Trtikova et al. 2010; Fischer et al. 2011). The fact that we did not find any relationship between number of flowers and elevation of origin or environmental factors related to elevation suggests that, despite the decreased biomass and maximum height in colder environments, reproductive output is not affected at higher elevations, and genetic variability in number of flowers among populations must therefore be due to other factors.

The near absence of correlations between plasticity (measured as the coefficient of variation of the population mean trait response across gardens) and elevation, temperature and precipitation of origin suggests that phenotypic plasticity evolves independently of these factors. This challenges propositions and findings that phenotypic plasticity has evolved especially in spatially or temporally heterogeneous environments shown to increase with elevation (Schlichting 1986; Körner 2003; Van Kleunen and Fischer 2005; Banta et al. 2007). However, a relationship between plasticity and elevation of origin was also absent in specific leaf area of *C. thyrsoides* for a partly overlapping set of populations from the Swiss Alps (Scheepens et al. 2010), as well as in a reciprocal transplantation experiment along two mountain slopes with *Scabiosa columbaria* (Pluess et al. 2012). Likewise, no increased plasticity was apparent in populations from the upper edge of the distribution of *Erigeron annuus* (Trtikova et al. 2010). In these studies, the elevational range of the sampled populations may have been too small and positioned at too high an elevation to observe increasing plasticity with elevation. Thus, since genetic variability in plasticity varies widely among species and traits, it still seems too early to propose a general pattern of increased plasticity in highest elevation alpine plants or with elevation in general (Van Kleunen and Fischer 2005).

Summary and conclusions

Our results showed that the phenology of *C. thyrsoides* was strongly advanced at lower elevations compared to higher elevations, which indicates an adaptive response to changing temperature. However, advanced flowering did not lead to the maintenance of fitness. We suggest that an accelerated flowering phenology at lower elevations caused this fitness reduction and, therefore, that the plastic response is not adaptive outside the species' native range. If global warming changes its habitat, *C. thyrsoides* may not be able to respond sufficiently to this new environment.

We also found that phenotypic variability in *C. thyrsoides* results from a combination of plastic responses as well as genetic effects, albeit to different extents depending on the trait. Some of the genetic differentiation among populations may be due to local adaptation, as suggested by correlations with elevation of origin and mean temperature. Genetic adaptation to future climatic conditions, although

slow, may aid survival of populations, whereas phenological and other traits that are controlled largely plastically may show maladaptive responses to novel conditions. We conclude from our results that the frequently reported phenological shift in plant species as a response to global warming may include heavy fitness costs that may hamper their survival in a warmer future.

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