Plastic Responses to Elevated Temperature in Low and High Elevation Populations of Three Grassland Species

Esther R. Frei*, Jaboury Ghazoul, Andrea R. Pluess

Department of Environmental Systems Science, Ecosystem Management, ETH Zurich, Zurich, Switzerland

Abstract

Local persistence of plant species in the face of climate change is largely mediated by genetic adaptation and phenotypic plasticity. In species with a wide altitudinal range, population responses to global warming are likely to differ at contrasting elevations. In controlled climate chambers, we investigated the responses of low and high elevation populations (1200 and 1800 m a.s.l.) of three nutrient-poor grassland species, Trifolium montanum, Ranunculus bulbosus, and Briza media, to ambient and elevated temperature. We measured growth-related, reproductive and phenological traits, evaluated differences in trait plasticity and examined whether trait values or plasticities were positively related to approximate fitness and thus under selection. Elevated temperature induced plastic responses in several growth-related traits of all three species. Although flowering phenology was advanced in T. montanum and R. bulbosus, number of flowers and reproductive allocation were not increased under elevated temperature. Plasticity differed between low and high elevation populations only in leaf traits of T. montanum and B. media. Some growth-related and phenological traits were under selection. Moreover, plasticities were not correlated with approximate fitness indicating selectively neutral plastic responses to elevated temperature. The observed plasticity in growth-related and phenological traits, albeit variable among species, suggests that plasticity is an important mechanism in mediating plant responses to elevated temperature. However, the capacity of species to respond to climate change through phenotypic plasticity is limited suggesting that the species additionally need evolutionary adaptation to adjust to climate change. The observed selection on several growth-related and phenological traits indicates that the study species have the potential for future evolution in the context of a warming climate.

Introduction

Global annual mean temperature has risen by approximately 0.85°C over the past century and climate scenarios project a further warming of 2 to 4°C for the 21st century [1]. This rapid temperature increase and the concomitant alterations of other environmental factors, such as higher levels of atmospheric CO2 and changing precipitation regimes, will drastically alter living conditions for plant and animal species across the globe and affect individuals, populations and communities [2–5]. Temperature is one of the main factors determining plant physiology and performance, and as a consequence, strongly influences the geographic distribution of plants [6,7]. Therefore, climate warming has caused latitudinal and altitudinal range shifts of many plant species worldwide and further shifts are expected [2,3]. Along elevation gradients, a considerable increase in number of plant species on alpine summits indicates an upward shift of species’ ranges [8–12]. However, the migration potential might be inferior to the rapid rate of current climate change, which is expected to render many species unable to track the climate they are currently adapted to [13,14]. Continuing habitat loss and fragmentation further exacerbate migration by impeding gene flow [15,16] and threaten the persistence of many species [17,18]. Alternatively to shifts in abundance and distribution, plants may persist in a changing climate through evolutionary adaptation and phenotypic plasticity [19–22]. Several studies provide evidence of climate driven population differentiation [23–26]. Thus, temperature and climate warming might be strong selective agents leading to the adaptive evolution of key plant traits [27,28]. In contrast to evolutionary adaptation, which requires several generations, current phenotypic plasticity in plants, the ability of a genotype to change its phenotype in response to different environments [29], allows short-term responses to rapid warming [21,30,31]. Among the best documented plastic responses to climate change are shifts in phenology observed in many species across the globe [2,3,32]. Although these meta-analyses are mainly based on genetically unstructured samples, which could not disentangle genetic and plastic changes, studies in phenological garden networks on structured samples show similar results [33,34]. Besides plastic shifts in phenology, several studies have documented morphological and physiological trait plasticity to temperature changes, reviewed in [35], and specifically plastic...
responses to elevated temperature, such as decreased seed dormancy [36,37] or reduced seed longevity [38].

Adaptive plasticity is the ability of an individual genotype to express phenotypes, which enhance fitness in response to variation in selection [21,31,39]. This is likely facilitating species persistence under climate change and assisting rapid evolutionary adaptation [40–44]. While numerous studies reported adaptive plasticity to different environmental conditions, such as shade [45], drought [46,47] and flooding [48], empirical evidence for adaptive plastic responses to climate change is still scarce, but see [49,50]. The capacity for plastic responses is supposed to vary due to different physiological thresholds, such as temperature and precipitation tolerance. Consequently, trait plasticity generally differs among species, and might even vary among populations from contrasting habitats across a species’ range [50,51]. Higher trait plasticity would be an advantage for plants experiencing greater spatial and temporal habitat heterogeneity because it allows them to maximize their fitness under different environmental conditions [39,52,53].

Mountain ecosystems are characterised by steep climate gradients and by increased environmental variation over short spatial and temporal scales at high as compared to nearby low elevation sites [54]. Because of the greater habitat heterogeneity we hypothesize that high elevation plants exhibit greater trait plasticity than their low elevation congeners. Varying climate conditions along altitudinal gradients might also result in different selection pressures that shape intraspecific trait adaptation to elevation. Little is known about how the adaptive potential of plants and their ability to respond to climate change through phenotypic plasticity shape phenotypic variation along altitudinal gradients. Transplant experiments in the Pyrenees and the Australian Alps revealed genetic differentiation but also trait plasticity [55,56]. In the Swiss Alps Frei et al. [57] described plasticity in reproductive phenology as an important plant response to transplantation to warmer low elevation sites. However, since these experiments were performed along natural elevation gradients, they were not able to separate the effects of temperature and warming from the influence of concomitant climate factors.

In this study we grew plants in controlled climate chambers and investigated responses of low (1200 m a.s.l.) and high (1800 m a.s.l.) elevation populations of three montane grassland species, *Trifolium montanum* L., *Ranunculus bulbosus* L. and *Briza media* L. to experimental warming. Plants were grown under elevated temperature, corresponding to predicted future conditions under climate change, and in control treatments corresponding to current ambient conditions at the plant origin whereas all other environmental parameters were kept identical. We measured growth-related, reproductive and phenological traits of the experimental plants and assessed differences in trait plasticity of low and high elevation populations. Using phenotypic selection analyses [58], we examined whether trait values and plasticities are under selection. Specifically, we addressed the following questions: (i) How are plant traits and fitness affected by elevated temperature? (ii) Does the degree of trait plasticity vary between low and high elevation populations? (iii) Are traits under selection? Does the strength or direction of selection differ between ambient and elevated temperature conditions? (iv) Does variation in temperature impose selection on trait plasticity? Studying current levels of genetic differentiation and variation in trait plasticity in response to elevated temperature will lead to a better understanding of future plant responses to predicted climate change.

Materials and Methods

Study Species

The common perennial species *Trifolium montanum* L., *Ranunculus bulbosus* L. and *Briza media* L. were selected for this study based on their co-occurrence in semi-dry nutrient-poor calcareous grasslands and their similar altitudinal range from 400 to 2000 m a.s.l [59]. The three species differ in phenology, pollination syndromes and reproduction: *Trifolium montanum* is an insect-pollinated late-flowering herb, *R. bulbosus* an insect-pollinated early-flowering herb and *B. media* a wind-pollinated grass.

The leguminous *Trifolium montanum*, which forms a symbiosis with soil rhizobia, produces 1–5 flowering shoots from June to July. A shoot bears 1–6 inflorescences with c. 150 yellow flowers [62]. The seeds lack dispersal aids and are therefore expected to be mainly gravity dispersed. After a period of aestivalisation, a new rosette is formed in early autumn [63,64].

*B. media* reproduces clonally and sexually with each ramet potentially forming one flower stalk [65]. The flowers emerge from June to July, have large feathery stigmas, and mature into indehiscent fruits that disperse next to the mother plant mainly by gravity [65]. Long-distance seed dispersal by grazing animals has also been observed [66].

Plant Material

In summer 2008, seeds were collected in seven to thirteen population pairs per species across the Swiss Alps (Table S1). For seed sampling, permits were obtained from the managers of the respective sites. Each population pair consisted of one population in the centre of the species range at c. 1200 m a.s.l. (1095–1275 m a.s.l.) and another close to the upper range limit at c. 1800 m a.s.l. (1720–1860 m a.s.l.), hereafter referred to as low and high elevation populations respectively. The vertical distance of 600 m between these elevations approximates a difference in annual mean temperature of 4°C [54] corresponding to the expected temperature rise of a typical climate change scenario for the year 2100 [1]. The horizontal distance between the two populations of a pair was 1–18 kilometres. Seeds were sampled from at least ten maternal plants in each population, air-dried and stored in paper bags at 4°C.

In spring 2009, seeds were germinated in a greenhouse. After four to six weeks, seedlings were planted into individual pots (800 cm³), filled with a 3:2 mixture of nutrient-poor commercial soil and sand. Pot positions were randomized weekly. From October 2009 to April 2010, the plants were overwintered outdoors at Davos (1500 m a.s.l.) to support vernalisation processes.

Climate Chamber Experiment

In May 2010, a controlled climate chamber experiment was established to test the responses of plants to current ambient temperature, corresponding to the temperature at their altitude of origin, and to elevated temperature, corresponding to 4°C higher temperature as compared to current temperature at their altitude of origin. The lowest temperature treatment (hereafter *T_min*) was ambient temperature for plants of high elevation origin (1800 m
Assessment of Plant Traits

In May 2010 and at the end of the growing season (October 2010), number of leaves (respectively ramets for B. media) and length of the longest leaf of each plant were measured. To obtain a measure for growth rate, the increment of number of leaves (respectively ramets for B. media) was calculated. In September 2010, one mature leaf per individual plant was sampled to assess specific leaf area (SLA). Immediately after sampling, the leaf blades were scanned (HP AllInOne colour Scanner, Hewlett-Packard GmbH, Dubendorf, Schweiz) and their areas were determined with LAMINA version 1.0.2 [68]. The scanned leaf blades were oven-dried at 60°C for 48 h and weighed. To determine SLA, we divided the leaf area by the dry weight of the leaf blade [69]. From May to July 2010, 75% of T. montanum and 84% of R. bulbosus individuals flowered. Due to the low flowering rate in B. media (8%), reproductive and phenological traits were only assessed for the two herbs. The phenological development was monitored twice a week to record the Julian Day (JD) of appearance of the first flower bud and the first open flower of each individual. In August, flowers of R. bulbosus and inflorescences of T. montanum were counted and the reproductive biomass (i.e. flower stalks and flowers) was harvested.

In October 2010, all plants were harvested and oven-dried. Above-ground (i.e. reproductive organs and leaves) and below-ground biomass of T. montanum and R. bulbosus were measured separately. Subsequently, biomass partitioning (i.e. above-ground biomass as a proportion of total biomass) and reproductive allocation (i.e. the proportion of reproductive biomass on total above-ground biomass) were calculated. In B. media, only above-ground biomass was assessed because we were not able to remove all soil sticking to the fine root system without considerable loss of root biomass.

Statistical Analyses

We first tested for effects of elevated temperature and genetic differentiation on phenotypic trait variation. Then, we conducted two sets of selection gradient analyses where we tested for selection on plant traits as well as for selection on trait plasticities in response to elevated temperature. All statistical analyses were performed with the statistical software R [70]. The residuals were checked for deviations from the model assumptions and the data was transformed if necessary [71]. To account for multiple tests we applied a Bonferroni correction within species whenever applicable.

We performed nested mixed-model analyses of covariance (ANCOVA) for each species separately to analyse the effects of elevated temperature and altitude of origin. The model consisted of the two fixed factors ‘altitude of origin’ and ‘temperature treatment’, the random factor ‘population’ and the two interaction terms ‘temperature treatment × altitude of origin’ and ‘temperature treatment × population’. A significant ‘altitude of origin’ effect (GAlt) indicates genetic differentiation between low and high elevation populations. A significant ‘temperature treatment’ effect (ETemp) indicates trait variation due to different environmental conditions i.e. phenotypic plasticity in a measured trait. A significant ‘temperature treatment × altitude of origin’ interaction (GAlt×ETemp) indicates differences in plasticity between the altitudes of origin. The ‘altitude of origin’ was tested on the population level and the ‘temperature treatment’ on the interac-

| Table 1. Daily cyles of the climate chamber program for the three temperature treatments Tmin, T and Tplus reflecting July outdoor temperatures at 1800 m, 1200 m and 600 m a.s.l. |

| Interval | Running time (h) | Temperature (°C) | Relative humidity (%) | Illumination (klx) |
|----------|-----------------|-----------------|----------------------|-------------------|
|          |                 | Tmin  | T    | Tplus | T    | Tplus | T    | Tplus | T    | Tplus | T    | Tplus | T    | Tplus |
| 1        | 6               | 8     | 12   | 16    | 72   | 0     | 72   | 0     | 72   | 0     | 72   | 0     | 72   | 0     |
| 2        | 2               | 6     | 10   | 14    | 84   | 0     | 84   | 0     | 84   | 0     | 84   | 0     | 84   | 0     |
| 3        | 5               | 6     | 9    | 13    | 84   | 0     | 84   | 0     | 84   | 0     | 84   | 0     | 84   | 0     |
| 4        | 1               | 5     | 18   | 22    | 26   | 55    | 26   | 55    | 26   | 55    | 26   | 55    | 26   | 55    |
| 5        | 2               | 2     | 19   | 23    | 27   | 51    | 27   | 51    | 27   | 51    | 27   | 51    | 27   | 51    |
| 6        | 6               | 2     | 18   | 22    | 26   | 51    | 26   | 51    | 26   | 51    | 26   | 51    | 26   | 51    |
| 7        | 4               | 10    | 14   | 18    | 63   | 1     | 63   | 1     | 63   | 1     | 63   | 1     | 63   | 1     |

Parameters were changed gradually to reach the set value at the end of the running time of each interval. Relative humidity and light regime were kept the same in all temperature treatments.

DOI:10.1371/journal.pone.0098677.t001
tion of 'temperature treatment × population' while all other terms were tested on the residuals. Besides direct responses to the environment, our measurements of plasticity might also be influenced by ontogenetic drift since the analyses did not account for plant size [72]. By including initial plant size [length of the longest leaf] as a covariate in the models we account for possible maternal effects [73].

We conducted phenotypic selection analyses on trait values in each temperature treatment to assess whether traits were under selection [58, 74]. Selection gradients were calculated based on regressions of relative fitness, measured as total biomass (T. montanum and R. bulbosus) and above ground biomass (B. media), on standardized trait values of individual plants. A trait was under positive selection if trait values were positively correlated with genotype fitness. To test whether selection differed between treatments, we calculated ANCOVAs of relative fitness in which we included the trait as a covariate and its interaction with the temperature treatment [48].

Phenotypic selection analyses on trait plasticities were performed to test whether trait plasticity itself was under selection, i.e. if plasticity was positively correlated with overall fitness, indicating adaptive plasticity. Selection gradients on trait plasticity were determined by regressing average fitness of a population on population trait values averaged over both treatments [i.e. elevation of the reaction norm] and on population values of plasticity [i.e. steepness of the reaction norm] [75, 76]. The elevation of the reaction norm was included to disentangle the fitness effect of the average value from the fitness effect of plasticity [77]. As proxies for fitness, we used number of inflorescences (T. montanum and R. bulbosus), total biomass (T. montanum and R. bulbosus) and above ground biomass (B. media). For both sets of selection gradient analyses, regression coefficients were standardized by expressing them in units of 1 SD to allow comparisons between traits and fitness measures.

Results

Environmental and Genetic Effects on Plant Variation

The model analyses allowed us to explain trait variation due to environmental effects of elevated temperature (ETemp) and genetic differentiation (GAlt), as well as their interactions (GAlt × ETemp). The random term population nested within altitude of origin had differentiation (GAlt), as well as their interactions (GAlt × ETemp) responded to elevated temperature (ETemp: \( P<0.001 \); Table 2; Fig. 1) and longer leaves \( (G_{Alt}: P=0.005) \) than high elevation plants whereas growth rate, SLA and the proportion of above-ground biomass did not differ between altitudes of origin \( (P>0.9) \). Budding and flowering started earlier in low as compared to high elevation plants \( (G_{Alt}: P<0.043; \) Table 3, Fig. 2). Number of flowers and reproductive allocation did not differ between low and high elevation plants \( (G_{Alt}: P>0.9) \).

Genetic differentiation in traits and plasticity. In T. montanum, plants of low elevation origin produced almost twice as much biomass \( (G_{Alt}: P<0.001; \) Table 2, Fig. 1) and longer leaves \( (G_{Alt}: P=0.005) \) than high elevation plants whereas growth rate, SLA and the proportion of above-ground biomass did not differ between altitudes of origin \( (P>0.9) \). Bud initiation and anthesis were advanced under elevated temperature \( (G_{Alt} \times E_{Temp}: P>0.1; \) Tables 2 and 3; Figs. 1 and 2). Plasticity of total biomass, growth rate, SLA and proportion of above-ground biomass as well as reproductive and phenological traits did not differ between low and high elevation populations \( (G_{Alt} \times E_{Temp}: P>0.1; \) Tables 2 and 3; Figs. 1 and 2). In R. bulbosus, low elevation plants produced longer leaves \( (G_{Alt}: P=0.005; \) Table 2; Fig. 1) and larger SLA \( (G_{Alt}: P<0.001) \) than high elevation plants whereas total biomass, growth rates and the proportion of above-ground biomass did not differ between low and high elevation plants \( (G_{Alt}: P>0.139) \). Low elevation plants tended to flower earlier than high elevation plants \( (G_{Alt}: P=0.074; \) Table 3, Fig. 2). Number of flowers, reproductive allocation and budding dates did not differ between low and high elevation plants \( (G_{Alt}: P>0.139) \). Furthermore, there were no significant differences in plasticity between low and high elevation populations neither in growth-related traits \( (G_{Alt} \times E_{Temp}: P>0.07; \) Table 2; Fig. 1) nor in reproductive and phenological traits \( (G_{Alt} \times E_{Temp}: P>0.9; \) Table 3; Fig. 2).

In B. media, low elevation plants produced more above ground biomass than high elevation plants \( (G_{Alt}: P=0.043; \) Table 2; Fig. 1). Leaf length, growth rate and SLA did not differ between low and high elevation plants \( (G_{Alt}: P=0.534) \). Furthermore, plasticity of leaf traits differed between altitudes of origin: leaf length and SLA increased in low elevation but decreased in high elevation plants under elevated temperature \( (G_{Alt} \times E_{Temp}: P<0.001; \) Table 2; Fig. 1). Plasticity of above ground biomass and growth rate did not differ between altitudes of origin \( (G_{Alt} \times E_{Temp}: P>0.504) \).

Selection Gradient Analyses on Traits and Plasticities

The selection gradient analyses on trait values in T. montanum revealed negative selection gradients for flower bud initiation and the appearance of the first flower averaged over both temperature treatments, which was reflected in a significant effect of these traits on fitness measured as total biomass \( (P<0.008; \) Table 4). In R. bulbosus, the selection analyses indicated a negative effect of SLA on fitness measured as total biomass \( (P=0.003; \) Table 4). In B. media, there was selection for higher growth rates averaged over both treatments \( (P<0.001) \). Selection gradient analyses on trait plasticities revealed no direct selection for plasticity in response to temperature in all traits and species \( (P=0.148; \) Table 5).
Table 2. Nested mixed-model ANCOVA for growth-related traits of *Trifolium montanum*, *Ranunculus bulbosus* and *Briza media* grown under ambient and elevated temperature.

| Trait                  | Biomass | Leaf length | Growth rate | SLA | Above-ground/total biomass |
|------------------------|---------|-------------|-------------|-----|-----------------------------|
|                        | d.f.    | MS          | F           | MS  | F                           | MS          | F       | MS          | F   |
| T. montanum            |         |             |             |     |                             |             |        |             |     |
| Initial plant size     | 1       | 19.31       | 76.26***    | 167.18 | 16.77***      | 4.74        | 14.36** | 105822.00   | 2.99 | 1.23 | 16.52***   |
| Temp                   | 1       | 7.07        | 27.91**     | 108.63 | 10.89**      | 4.16        | 12.59*  | 157334.00   | 4.45 | 0.73 | 9.83(*)    |
| Orig                   | 1       | 15.24       | 60.18***    | 198.74 | 19.93**      | 0.07        | 0.22    | 76416.00    | 2.16 | 0.49 | 6.57       |
|Pop[Orig]               | 18      | 0.38        | 1.52        | 11.47 | 1.15         | 0.50        | 1.53    | 47855.00    | 1.35 | 0.28 | 3.75***    |
| Temp × Orig            | 1       | 0.49        | 1.95        | 243.42 | 24.41***     | 0.33        | 0.99    | 38816.00    | 1.10 | 0.06 | 0.84       |
| Temp × Pop             | 18      | 0.32        | 1.25        | 7.21  | 0.72         | 0.35        | 1.06    | 43784.00    | 1.24 | 0.08 | 1.04       |
| Residuals              | 128     | 0.25        | 9.97        | 0.33  | 0.33         | 35383.00    | 0.07    | 0.07        |     |
| R. bulbosus            |         |             |             |     |                             |             |        |             |     |
| Initial plant size     | 1       | 15.99       | 22.61***    | 0.26  | 0.21         | 394.70      | 12.96** | 6230.20     | 5.16 | 0.00 | 0.13       |
| Temp                   | 1       | 1.06        | 1.50        | 10.56 | 8.49(*)      | 378.12      | 12.41*  | 2107.50     | 1.75 | 0.00 | 0.11       |
| Orig                   | 1       | 0.05        | 0.07        | 26.08 | 20.96**      | 504.64      | 16.56   | 30617.20    | 25.38*** | 0.03 | 0.77       |
| Pop[Orig]              | 12      | 0.88        | 1.24        | 1.18  | 0.95         | 66.72       | 2.19    | 86310.00    | 0.72 | 0.04 | 1.31       |
| Temp × Orig            | 1       | 0.49        | 0.70        | 0.22  | 0.17         | 18.98       | 0.62    | 101557.00   | 8.42(*)  | 0.01 | 0.28       |
| Temp × Pop             | 12      | 0.88        | 1.25        | 0.94  | 0.76         | 21.58       | 0.71    | 1267.10     | 1.05 | 0.02 | 0.46       |
| Residuals              | 47–59   | 0.71        | 1.24        | 30.47 | 0.89         | 1206.40     | 0.03    |             |     |
| B. media               |         |             |             |     |                             |             |        |             |     |
| Initial plant size     | 1       | 4.33        | 86.02***    | 54.40 | 14.45***     | 9.19        | 0.95    | 0.22        | 5.30(*)  |
| Temp                   | 1       | 1.74        | 34.68***    | 14.84 | 3.94*        | 132.64      | 13.71*  | 0.04        | 0.87   |
| Orig                   | 1       | 1.29        | 25.66*      | 0.05  | 0.01         | 24.62       | 2.54    | 0.09        | 2.14   |
| Pop[Orig]              | 24      | 0.17        | 3.36***     | 5.56  | 1.48         | 15.46       | 1.60    | 0.04        | 0.89   |
| Temp × Orig            | 1       | 0.11        | 2.13        | 333.25 | 88.50***     | 1.62        | 0.17    | 1.31        | 31.99*** |
| Temp × Pop             | 24      | 0.03        | 0.67        | 1.91  | 0.51         | 12.45       | 1.29    | 0.04        | 0.99   |
| Residuals              | 259     | 0.05        | 3.77        | 9.68  | 0.04         |             |         |             |     |

d.f., degrees of freedom; MS, mean squares; F, F-values; Initial plant size, covariate; Temp, temperature treatment; Orig, altitude of population origin; Pop, population. Residual d.f. vary per trait due to missingness. Bonferroni-corrected significance levels within species are indicated by asterisks: ***P<0.001, **P<0.01, *P<0.05, (*)P<0.08.
doi:10.1371/journal.pone.0098677.t002
Table 3. Nested mixed-model ANCOVA for reproductive and phenological traits of *Trifolium montanum* and *Ranunculus bulbosus* grown under ambient and elevated temperature.

|                     | Number of flowers | Reproductive allocation | Budding start | Flowering start |
|---------------------|-------------------|-------------------------|---------------|-----------------|
|                     | d.f.              | MS          | F     | MS          | F     | MS          | F     | MS          | F     |
| T. montanum         |                   |             |       |             |       |             |       |             |       |
| Initial plant size  | 1                 | 64.76       | 47.07*** | 1.96        | 42.38*** | 2472.90     | 30.61*** | 17.43       | 36.37*** |
| Temp                | 1                 | 0.25        | 0.18  | 0.01        | 0.17   | 5641.00     | 6.98**  | 11.24       | 23.46*** |
| Orig                | 1                 | 6.33        | 4.60  | 0.12        | 2.55   | 7049.00     | 8.73*   | 10.79       | 22.51**  |
| Pop[Orig]           | 18                | 2.49        | 3     | 0.09        | 1.84   | 680.80      | 0.84    | 0.58        | 1.22    |
| Temp × Orig         | 1                 | 8.53        | 6.20  | 0.02        | 0.50   | 0.10        | 0.00    | 1.00        | 2.09    |
| Temp × Pop          | 17–18             | 0.96        | 0.70  | 0.03        | 0.71   | 340.00      | 0.42    | 0.23        | 0.47    |
| Residuals           | 77–128            | 1.38        | 0.05  |             |        | 807.80      |         | 0.48        |         |
| R. bulbosus         |                   |             |       |             |       |             |       |             |       |
| Initial plant size  | 1                 | 18.19       | 22.86*** | 0.12        | 8.82*  | 3312.60     | 23.61*** | 3925.90     | 26.58*** |
| Temp                | 1                 | 3.08        | 3.87  | 0.02        | 1.73   | 1188.00     | 8.47*** | 2238.00     | 15.15*** |
| Orig                | 1                 | 1.88        | 2.36  | 0.14        | 10.12  | 627.10      | 4.47    | 2197.50     | 14.88(*) |
| Pop[Orig]           | 12                | 0.97        | 1.23  | 0.02        | 1.27   | 391.80      | 2.79*1  | 219.70      | 1.49    |
| Temp × Orig         | 1                 | 0.69        | 0.86  | 0.01        | 0.43   | 16.90       | 0.12    | 3.30        | 0.02    |
| Temp × Pop          | 12                | 0.77        | 0.97  | 0.02        | 1.67   | 30.30       | 0.22    | 29.00       | 0.20    |
| Residuals           | 45–59             | 0.80        | 0.01  |             |        | 140.30      |         | 147.70      |         |

d.f., degrees of freedom; MS, mean squares; F, F-values; Initial plant size, covariate; Temp, temperature treatment; Orig, altitude of population origin; Pop, population. Residual d.f. vary per trait due to missingness. Bonferroni-corrected significance levels within species are indicated by asterisks: ***$P_{Bonf}$ < 0.001, **$P_{Bonf}$ < 0.01, *$P_{Bonf}$ < 0.05, (*)$P_{Bonf}$ < 0.08.

doi:10.1371/journal.pone.0098677.t003
Discussion

In the present study, we evaluated the effects of elevated temperature on phenotypic variation of the three perennial grassland species *T. montanum*, *R. bulbosus* and *B. media*. We investigated if trait plasticity varies between low and high elevation populations and if trait values and plasticities were under selection.

Plastic Responses to Elevated Temperature

Positive warming effects on several growth-related traits in all three species (Table 2) indicate that plant growth is constrained by low temperature conditions, which is common in temperate species [6]. In arctic and temperate zones plant species spend the majority of their life at mean temperatures below the growth optimum [78]. Thus, a moderate warming could enhance plant performance. Meta-analyses of in-situ warming experiments with arctic and alpine tundra species revealed biome-wide trends of increased vegetative growth, albeit variable among species [79,80]. The variable responses to elevated temperature of the three study species might be associated with differences in characteristics of below-ground organs. Besides the positive responses in the other growth-related traits, the nitrogen-fixing *T. montanum* showed a slight decrease in the proportion of above ground biomass. The observed greater investment in roots under elevated temperature is contrary to the general trend that allocation to root biomass increases with decreasing temperature at higher elevation [54]. However, it is in line with the findings of Roughley and Dart [81] who described a positive effect of soil temperature on the formation of rhizobia in *Trifolium subterraneum*. The increased number of rhizobia enhances nitrogen availability, which stimulates root growth. *Fabaceae*, such as *T. montanum*, might therefore gain a competitive advantage over non-nitrogen-fixing species under warmer climate conditions. Competition experiments with elevated CO₂, which has a similar positive effect on nitrogen fixation as elevated temperature, provide evidence for such a competitive advantage, reviewed in [82]. The less pronounced warming effect on growth-related traits in *R. bulbosus* could be related to the species-specific life-form. The nutrient-storing corm might allow a temperature independent formation of new above ground tissue after the period of summer aestivation and buffer against short-term climatic fluctuations as shown for *R. niveus* [83].

Preformation of flower buds might lead to a time lag in the reproductive response to warming potentially explaining why the experimental warming did not affect reproductive traits of *T. montanum* and *R. bulbosus* (Table 3). This phenomenon is relatively common among temperate herbaceous perennials [84] and has been documented for tundra species in in-situ warming experiments [79]. Thus, it might have required at least two growing seasons to provide a strong estimate of the effects of increased temperature on reproduction of the perennial study species. Although these species did not respond plastically to elevated temperature, they are likely to be affected by changes in other environmental factors (e.g. reduced precipitation and higher evapotranspiration) co-occurring in natural systems. Alternatively, the lack of reproductive responses could indicate that reproductive traits are genetically fixed suggesting that these species need to adjust to climate change through the relatively slow process of evolutionary adaptation [20,85]. In the context of rapid climate change, this process might be too slow to secure plant persistence [86–88]. Evidence for evolutionary adaptation to climate change is still scarce, and rapid evolution has been documented mainly in fast-growing annuals [27,89] but also in perennials [90].

Reproductive phenology of *T. montanum* and *R. bulbosus* was advanced under elevated temperature (Table 3) confirming the findings of other studies that these traits respond plasticity to temperature, e.g. [2,5,79,91–95]. Moreover, our findings are in line with the advanced reproductive phenology of the same species grown in a common garden transplant experiment [57]. The advanced spring phenology facilitates setting flowers and seeds before the hot and dry midsummer period. It might therefore enhance plant fitness, but also bears the risk of damage by late frost events, especially in high elevation populations [54,96,97]. Furthermore, plant-pollinator-interaction models indicate that pollinating insects might not keep up with shifted flowering periods [98], which might impede insect pollination and thereby reduce reproductive success [99,100].

In summary, low and high elevation populations exhibited plasticity to temperature in reproductive phenology and several growth-related traits. The advanced flowering and the generally enhanced growth, albeit variable among species, indicate the ability of these species to cope with climate warming at least in the short term.

Genetic Variation in Traits and Differences in Plasticity between Low and High Elevation Populations

We found only little genetic variation among populations within each altitudinal origin (Tables 2 and 3) suggesting small selection differences and high genetic connectivity. Indeed, a previous study on similar populations of the same three species using neutral molecular markers revealed intermediate genetic diversity but only low genetic differentiation among populations and assigned this to extensive historic gene flow [101]. The genetic differentiation between low and high elevation populations in some growth-related and phenological traits (Tables 2 and 3) indicated that selection in the past has acted differently on low and high elevation plants. Alternatively, the observed genetic differences might be due to neutral genetic processes [24,102]. However, maternal effects were accounted for by including initial plant size as a covariate in the models and genetic drift is unlikely to have acted in the same direction on all sampled populations since seeds were sampled from multiple population pairs distributed over the Swiss Alps.

In contrast to the hypothesis of greater plasticity at more heterogeneous high elevation sites, plasticity of leaf length was reduced in high elevation populations of *T. montanum* (Table 2; Fig. 1). Similarly, a study with seedlings of European deciduous tree species found that high elevation provenances exhibited less temperature-induced plasticity in growth and leaf phenological traits [103,104]. The authors argued that low plasticity at high elevations was a result of different directional selection for reduced temperature sensitivity and a stronger influence of photoperiodism, which may reduce the risk of damage by unpredictable late spring frost events [105]. Moreover, the greater plasticity of leaf length in low elevation plants might have been induced by higher levels of competition at these sites [106]. In *B. media*, plastic responses of leaf length and SLA to elevated temperature were positive in plants from warmer low elevations, but negative in
Plant Population Responses to Elevated Temperature

**Trifolium montanum**
- Number of flowers
  - Ambient: 8
  - Elevated: 7

**Ranunculus bulbosus**
- Altitude of origin
  - 1800: 7
  - 1200: 6

**Reproductive allocation (%)**
- Ambient: 35
- Elevated: 30

**Budding start (JD)**
- Ambient: 180
- Elevated: 155

**Flowering start (JD)**
- Ambient: 200
- Elevated: 175

Temperature treatment
plants from colder high elevations (Table 2; Fig. 1). In low elevation plants, elevated temperature induced longer leaves and greater SLA. Thus, the warming seems to have triggered a greater investment in vegetative growth. High elevation plants invested more in clonal propagation as can be concluded from their shorter and thicker leaves as indicated by the smaller leaf length and SLA in combination with an increase in number of ramets (details not shown). In-situ warming experiments showed similar response patterns, reviewed in [79]. The stronger growth response in low arctic plants was related to higher competition in these communities [107] whereas the greater reproductive response of high arctic plants was attributed to increased colonization efforts in these habitats where competition is less important [108].

In summary, plasticity differed between altitudes of origin in only a few traits and there was little genetic differentiation among populations within each altitudinal origin suggesting uniform responses to climate warming. Moreover, the high connectivity together with the moderate genetic differentiation between altitudes of origin might facilitate future adaptation to climate change.

Selection on Traits and their Plasticities

We found indication for selection on several growth-related and phenological traits (Table 4). In T. montanum, the two phenological traits budding and flowering start were under negative selection with respect to biomass; plants with earlier appearing flower buds and flowers ended up with higher biomass at the end of the growing season. Although we did not record leaf phenology, it is likely that the advanced flowering phenology was related to a similar advance in other spring phenophases [32] allowing these plants more time to accumulate biomass. In B. media, selection for higher growth rates can be explained by the fact that faster growing individuals acquired more above ground biomass [7]. Although plasticity to temperature was not adaptive in our experiment (Table 5), increased plant size under elevated temperature might eventually lead to higher fitness since flower bud preformation is common in perennials and sexual reproduction is often positively correlated with plant size [109]. Increased growth under warmer temperature might thus be beneficial for plant persistence in the longer term whereas the duration of our experiment might have been too short for observing fitness benefits of plasticity. The magnitude and even the direction of selection on a trait may differ for different components of fitness [74]. This might also have affected the results of our selection analyses and shows the need for more comprehensive fitness measures [110]. Furthermore, it is generally recommended to analyse phenotypic selection on trait means and their plasticities based on genotypic values [111] to avoid environmentally induced covariation [112]. However, our experimental design only allowed us to analyse selection based on individual values or population means, see [113]. This might have resulted in a loss of discriminative power because the observed variation is a combination of phenotypic plasticity and within population genetic variability. Thus, future selection studies at the genotype level are recommended to gain deeper insights into the mechanisms of plasticity in plant populations.

In summary, the absence of selection on phenotypic plasticity indicated that the observed trait plasticity was selectively neutral. Selection on several growth-related and phenological traits

Table 4. Selection gradients of growth-related and phenological traits of Trifolium montanum, Ranunculus bulbosus and Briza media under ambient and elevated temperature and F-values of ANCOVA testing for overall selection (Cov) and differences in selection between temperature treatments (Cov × Temp).

| Trait          | Selection gradients | F-Values of ANCOVA |
|----------------|---------------------|--------------------|
|                | ambient elevated    | Cov                | Cov × Temp |
| T. montanum    |                     |                    |
| Growth rate    | −0.320 0.009        | 0.15 4.57          |
| SLA            | −0.332 0.009        | 0.85 0.17          |
| Budding start  | −0.543 −0.170       | 10.07** 0.80       |
| Flowering start| −0.804 −0.297       | 19.58*** 1.40      |
| R. bulbosus    |                     |                    |
| Growth rate    | −0.279 −0.071       | 4.88 1.35          |
| SLA            | −0.365 −0.309       | 12.68** 0.70       |
| Budding start  | −0.119 −0.145       | 0.94 0.04          |
| Flowering start| −0.236 −0.309       | 4.50 0.23          |
| B. media       |                     |                    |
| Growth rate    | 0.044 0.079         | 20.70*** 0.02      |
| SLA            | −0.040 0.025        | 0.38 3.38          |

Selection gradients are expressed as standardized regression coefficients after regression of the fitness measures total biomass (T. montanum, R. bulbosus) and above ground biomass (B. media) on the respective trait. All calculations are based on individual plant values. Bonferroni-corrected significance levels are indicated by asterisks: ***P < 0.001, **P < 0.01, *P < 0.05.

doi:10.1371/journal.pone.0098677.t004
suggested that there is potential for future evolution of mean trait values allowing the study species to adapt to elevated temperature provided there is sufficient genetic variation and heritability of the respective traits.

Conclusions

We investigated phenotypic variation in low and high elevation populations of nutrient-poor grassland species in response to elevated temperature. The three species exhibited trait plasticity with respect to temperature whereas genetic differentiation and differences in plasticity between low and high populations were less important. Plasticity in growth and flowering phenology determines the ability of the study species to respond to elevated temperature by buffering against detrimental effects of rapid climate change and allowing time for evolutionary adaptation. However, the capacity of species to respond to environmental changes through phenotypic plasticity is limited and plasticity alone might not be sufficient to cope with climate change. Thus, plants additionally need to adjust through the relatively slow process of evolutionary adaptation. Selection on several traits suggests that the three species have the potential for evolutionary changes, which might allow them to adapt to a future climate.

Supporting Information

Table S1 Sampling locations in the Swiss Alps of the 
Supporting Information

Conclusions

We investigated phenotypic variation in low and high elevation populations of nutrient-poor grassland species in response to elevated temperature. The three species exhibited trait plasticity with respect to temperature whereas genetic differentiation and differences in plasticity between low and high populations were less important. Plasticity in growth and flowering phenology determines the ability of the study species to respond to elevated temperature by buffering against detrimental effects of rapid climate change and allowing time for evolutionary adaptation. However, the capacity of species to respond to environmental changes through phenotypic plasticity is limited and plasticity alone might not be sufficient to cope with climate change. Thus, plants additionally need to adjust through the relatively slow process of evolutionary adaptation. Selection on several traits suggests that the three species have the potential for evolutionary changes, which might allow them to adapt to a future climate.

Supporting Information

Table S1 Sampling locations in the Swiss Alps of the 

Table 5. Selection gradients of trait plasticities in response to temperature in Trifolium montanum, Ranunculus bulbosus and Briza media.

| T. montanum | R. bulbosus | B. media |
|-------------|-------------|----------|
| Total biomass | Number of flowers | Total biomass | Number of flowers | Above ground biomass |
| Growth rate | 0.500 | 0.455 | 0.064 | 0.258 | 0.366 |
| SLA | 0.289 | 0.480 | –0.171 | 0.315 | – |
| Above-ground/total biomass | –0.497 | –0.189 | –1.334 | –0.450 | –0.577 |
| Budding start | 0.307 | 0.380 | 0.039 | 1.156 | – |
| Flowering start | 0.307 | 0.380 | 0.039 | 1.156 | – |

Selection gradients are expressed as standardized regression coefficients. All values are based on population means. Fitness measures are total biomass and number of flowers (T. montanum, R. bulbosus) and above ground biomass (B. media). Non of the selection gradients was significant.

doi:10.1371/journal.pone.0098677.t005

Table S2 Means and standard errors (SE) grouped by altitude of population origin and temperature treatment for growth-related traits of Trifolium montanum, Ranunculus bulbosus and Briza media.

Table S3 Means and standard errors (SE) grouped by altitude of population origin and temperature treatment for reproductive and phenological traits of Trifolium montanum and Ranunculus bulbosus.

Acknowledgments

The authors thank M. Beck, M. Freci, S. Frischknecht, T. Hahn, M. Heggi, D. Kurth, J. Leuenberger, M. Leuzinger, M. Macai and P. Matter who helped collecting seeds, cultivating and harvesting plants. They are grateful to F. Grasein, M. Kalisch, M. van Kleunen and L. Till-Bottraud for statistical advice as well as to E.S. Freci, M. Heggi and C.J. Kettle for valuable comments on the manuscript. The authors thank the editor and two anonymous reviewers for helpful comments.

Author Contributions

Conceived and designed the experiments: ERF ARP JG. Performed the experiments: ERF. Analyzed the data: ERF ARP. Contributed reagents/materials/analysis tools: ERF ARP. Wrote the paper: ERF JG ARP.

References

1. IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK et al., editors. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press. 1535 p.
2. Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37–42.
3. Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, et al. (2003) Fingerprints of global warming on wild animals and plants. Nature 421: 57–60.
4. Walker MD, Wahren CH, Hollister RD, Henry GHR, Ahlquist LE, et al. (2006) Plant community responses to experimental warming across the tundra biome. Proceedings of the National Academy of Sciences of the United States of America 103: 1342–1346.
5. Walther GR, Post E, Convey P, Menzel A, Parmesan C, et al. (2002) Ecological responses to recent climate change. Nature 416: 389–395.
6. Hopkins WG, Huner NPA (2000) Introduction to plant physiology. Hoboken, New Jersey, United States: John Wiley & Sons.
7. Lambers H, Chapin FS III, Pons TL (2008) Plant physiological ecology. New York, United States: Springer.
8. Walther GR, Beisner S, Burca CA (2003) Trends in the upward shift of alpine plants. Journal of Vegetation Science 16: 541–548.
9. Freci E, Bodin J, Walther GR (2010) Plant species’ range shifts in mountainous areas – all uphill from here? Botanica Helvetica 120: 117–128.
10. Gottfried M, Pausl H, Futschik A, Akhalkatsi M, Baranecok P, et al. (2012) Continent-wide response of mountain vegetation to climate change. Nature Climate Change 2: 111–115.
11. Engler R, Randin CF, Thuiller W, Dullinger S, Zimmermann NE, et al. (2011) 21st century climate change threatens mountain flora unequally across Europe. Global Change Biology 17: 2330–2341.
12. Lensoi J, Gregout JC, Maruet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. Science 320: 1768–1771.
13. Huntley B (1991) How plants respond to climate change: migration rates, individualism and the consequences for plant communities. Annals of Botany 67: 15–22.
14. Davis MB, Shaw RG (2001) Range shifts and adaptive responses to quaternary climate change. Science 292: 673–679.
15. Collingham YC, Huntley B (2000) Impacts of habitat fragmentation and patch size upon migration rates. Ecological Applications 10: 131–144.
16. Honnay O, Verheyen K, Butaye J, Jacquemyn H, Bossuyt B, et al. (2002) Possible effects of habitat fragmentation and climate change on the range of forest plant species. Ecology Letters 5: 525–530.
17. Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, et al. (2004) Extinction risk from climate change. Nature 427: 145–148.
18. Lesica P, McCune B (2004) Decline of arctic-alpine plants at the southern margin of their range following a decade of climate warming. Journal of Vegetation Science 15: 679–690.
19. Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature 470: 479–485.
20. Jump AS, Penuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8: 1010–1020.
21. Nicotra AB, Akin OK, Bonser SP, Davidson AM, Finnegan EJ, et al. (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15: 604–692.
22. Gienapp P, Teplitsky C, Alho JS, Mills JA, Merila J (2008) Climate change and evolution: disentangling environmental and genetic responses. Molecular Ecology 17: 167–178.
23. Joshi J, Schmid B, Caldeira M, Dimitrakopoulos P, Good j, et al. (2001) Local adaptation enhances performance of common plant species. Ecology Letters 4: 536–544.
24. Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27: 237–268.
25. Macel M, Lawson CS, Mortimer SR, Smilauerova M, Bischoff A, et al. (2007) Climate vs. soil factors in local adaptation of two common plant species. Ecology 88: 424–433.
26. Becker U, Colling G, Dostal P, Jakobsson A, Mathies D (2006) Local adaptation in the monocarpic perennial Cactina vulgaris at different spatial scales across Europe. Oecologia 150: 506–518.
27. Franks SJ, Sin S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences of the United States of America 104: 1278–1282.
28. Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. Ecology 86: 1704–1714.
29. Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115–155.
30. Valladeres F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and ecological applications. Journal of Ecology 94: 1105–1116.
31. Ghahambar CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21: 394–407.
32. Mauzel A, Spalding TP, Estrella N, Koch E, Aasa A, et al. (2006) European phenological response to climate change matches the warming pattern. Global Change Biology 12: 1969–1976.
33. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
34. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
35. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
36. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
37. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
38. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
39. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
40. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
41. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
42. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
43. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
44. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
45. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
46. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
47. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
48. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
49. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
50. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
51. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
52. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
53. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
54. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
55. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
56. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
57. van Vliet AJH, de Groot RS, Bellens Y, Braun P, Bruggen R, et al. (2003) The role of phenotypic plasticity in plants. Research review. New Phytologist 166: 49–60.
94. De Frenne P, Brunet J, Shevtsova A, Kolb A, Graae BJ, et al. (2011)
93. Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, et al. (2009)
92. Parmesan C (2007) Influences of species, latitudes and methodologies on
91. Menzel A (2003) Plant phenological anomalies in Germany and their relation
90. Didiano TJ, Turley NE, Everwand G, Schaefer H, Crawley MJ, et al. (2014)
89. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species,
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}:
79. Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, et al. (1999)
80. Elmendorf SC, Henry GHR, Holyst RE, Bjork RG, Bjorkman AD, et al. (2012)
88. Bürgers R, Lynch M (1995) Evolution and extinction in a changing environment
86. Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to
85. Pulido F, Berthold P (2004) Microevolutionary response to climatic change. In:
83. Molau U (1997) Responses to natural climatic variation and experimental
82. Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes
81. Roughley RJ, Dart PJ (1970) Growth of \textit{Polygonum viviparum}: