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Effects of temperature on performance and phenotypic selection on plant traits in alpine *Ranunculus acris*

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Abstract Discovering temperature effects on the performance of tundra plants is important in the light of expected climate change. In this 4-year study on alpine *Ranunculus acris*, I test the hypothesis that temperature influences flowering phenology, reproductive success, growth, population dynamics, and phenotypic selection on quantitative traits, by experimental warming using open-top chambers (OTCs). Warming significantly advanced flowering phenology in only one season. Seed number and weight were significantly increased by warming during the first three seasons, but not in the fourth. Plants inside OTCs produced bigger leaves than control plants in the fourth season, but leaf number was unaffected by the OTC treatment. Despite increased seed number and weight, the density of flowering plants decreased inside OTCs compared to control plots, possibly because of a higher graminoid cover inside OTCs. Phenotypic-selection regression showed a significant selection differential and gradient in the direction of larger leaf sizes in control plants, whereas no selection on leaf size was detected on warmed plants. The direction and strength of selection on flowering time, flower number, and leaf number did not differ between control and warmed plants. The results suggest that increased reproductive output of *R. acris* may not be sufficient to maintain current population density under a denser vegetation cover.

Key words Climate change · Growth · Flowering phenology · Population dynamics · Seed production

Introduction

Temperature conditions may impose severe constraints on the performance of plants in alpine environments (Billings 1987) through many processes. First, the activity of pollinators is strongly affected by temperature conditions because of thermal constraints on the flight activity of insects (McCall and Primack 1992; Totland 1994a; Bergman et al. 1996). Second, the processes of pollen tube germination and growth, ovule fertilization, and seed maturation may be hindered or delayed by too low temperatures (Corbet 1990; Totland 1997a, 1997b). Third, since reproductive output of plants often depends on plant size (Samson and Werk 1986; Shipley and Dion 1992), temperature constraints on growth may indirectly affect reproductive output through plant-size effects on, for example, flower and ovule number, and resource allocation and availability. Fourth, onset of flowering in alpine and arctic plants is dependent on prefloration temperature conditions in many species (Thórhallsdóttir 1998), and since flowering time may be critical for reproductive success in several species (Molau 1993), temperature conditions in alpine habitats may affect fitness of plants via their effects on flowering time. Temperature conditions may thus function as a strong selective agent through their effects on numerous fitness-related traits, such as plant size, ovule number, flower number, and flowering time.

As indicated by Mitchell-Olds and Shaw (1987), Wade and Kalisz (1990), Rausher (1992), and Mauricio and Mojonier (1997), the relationship between a given trait and fitness may differ across an environmental mosaic. In alpine habitats, the climate conditions that influence plants in a population are likely to vary substantially at several scales, since spatial differences in temperature conditions across scales from a few meters to several kilometers are likely to occur due to variation in for example altitude, exposure, snow-melt time, and soil moisture. The effect of temperature on selection pathways depends on the impact and consistency of

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temperature effects on fitness-related traits both in space and time. If, for example, there is a strong relationship between flower number and fitness for plants situated in relatively warm parts of a population, whereas no such, or even a negative, relationship exists between flower number and fitness in cooler parts of the population, then the ability of selection to promote the evolution of flower number may be weak, since under such conditions fitness relationships vary spatially (e.g., Galloway 1995; Stanton and Galen 1997). Moreover, temperature effects on reproductive success may vary between seasons due to large annual fluctuation in temperature conditions (Philipp et al. 1990). If such temporal differences in temperature produce differences in the strength or direction of fitness relationships, they are likely to weaken the process of selection in creating adaptive changes.

Discovering effects of temperature on the performance of alpine plant species has become increasingly important in the light of the expected global warming, as a result of increased accumulation of greenhouse gases in the atmosphere. General circulation models predict that warming will be most pronounced in alpine and arctic areas (Maxwell 1992), and due to the proposed strong effects of temperature conditions on alpine and arctic plants (Billings 1987), populations in these ecosystems may be strongly affected by global warming, through environmental effects on establishment, growth, and reproduction (e.g., Callaghan and Jonasson 1995; Chapin and Körner 1995; Chapin et al. 1995; Harte and Shaw 1995; Henry and Molau 1997; Totland and Nylén 1998).

One way in which populations in these ecosystems can endure altered environmental conditions is by evolutionary adjustments that make plants better adapted to the new environmental conditions (McGraw and Fetcher 1992; Geber and Dawson 1993; McGraw 1995). A first step towards an understanding of whether populations can adapt to new environmental conditions is to examine whether relationships between traits and fitness are different in natural and manipulated environmental conditions resembling those predicted for the future. However, despite their potentially great importance, evolutionary responses of tundra plant species to an altered selective regime under global warming have received little attention.

In this study I experimentally increased growing season temperature in an alpine population of the perennial insect-pollinated herb *Ranunculus acris* over four seasons and examined whether plant characters responded to this environmental perturbation, and whether responses differed among seasons. In addition, during the fourth season, I examined whether fitness relationships were affected by environmental conditions, using phenotypic-selection regressions (Lande and Arnold 1983). This technique can detect environment-specific differences in the relationship of the characters to fitness, and may indicate whether selection on characters is mediated by environmental conditions (Mitchell-Olds and Shaw 1987), a prerequisite for evolutionary

adaptations to changed environmental conditions. Specifically, I address the following questions:

1. Does experimental warming during the growing season affect flowering phenology, growth, and reproduction of *R. acris*?
2. If so, do effects of warming vary among growing seasons?
3. Does warming affect the abundance of flowering plants over a 4-year period?
4. Do the strength and direction of phenotypic selection on flowering time, flower number, and plant size differ between experimentally warmed and control plots?

Materials and methods

Study site and species

Ranunculus acris L. is a perennial geophyte of circumpolar distribution. The studied plants are of the taxon *R. acris* subsp. *acris* var. *pumilus* Wahlenb. At Finse in south-west Norway, where the study was done, *R. acris* grows in dry and wet meadows, on river banks, and stony slopes, but it is most abundant on medium dry meadows in early melting snow-beds. Although *R. acris* is known to reproduce vegetatively by rhizomes, excavations of several root systems suggested that this occurs to a very limited extent in plants at Finse. Plants produce *c.* four basal leaves each season. The five-petaled, yellow, hermaphroditic flowers are born on an erect stem. Most plants (74%) at the study site produce only one flower, whereas 22% produce two, and 4% produce three flowers in a given year. Flowering of *R. acris* begins early after snowmelt (*c.* 3 weeks, Totland 1993). Flowers are self-incompatible, and thus require cross-pollination by insects for seed production (Totland 1994b). Flies of the families Muscidae and Anthomyiidae are the main pollinators of *R. acris* at Finse (Totland 1993). A single flower-visit by these flies results in the fertilization and maturation of *c.* 17% of the ovules in a flower (Totland 1994b). A flower contains *c.* 30 carpels which after fertilization turn into indehiscent achenes (hereafter termed seed).

This study was done on a population of *R. acris* situated on a stable solifluction mat on the south face of Sandalsnuten (60°7'N, 7°32'E), *c.* 2.5 km north of Finse railway station, at *c.* 1500 m elevation. Monthly average temperatures at 1224 m elevation at Finse for June, July, August, and September, during 1960–1990 were 5.0, 7.0, 6.8, and 3.0°C, respectively. During July and August the average daily temperature at 1224 m elevation was 8.4° in 1994, 7.4° in 1996, and 10.4° in 1997. In 1995 temperature was not recorded during August. Average daily temperature during July in this year was 6.7°C. The snow cover in the study area disappeared between mid and late June in the years of study. The vegetation in the study area is meadow-like, and abundant species are *Carex bigelowii*, *Salix herbacea*, *Bartsia alpina*, and *R. acris*.

Open-top chamber experiment

I used open-top chambers (OTCs, Marion et al. 1997) to study the impacts of temperature on fitness-related traits in *R. acris*. Each OTC covered a basal area of 60 × 60 cm, and was 40 cm high with 80° inwardly declined sides. They are made of 3 mm thick UV-resistant polycarbonate which reduces photosynthetic active radiation by 12% (measured during the fourth season with a LI-250 quantum sensor; Li-cor Inc., Lincoln, Neb., USA). OTCs increased daily average temperature by 2.3°C at 5 cm above ground, by 3.1°C at ground level, and by 0.8°C at 5 cm soil depth, above controls (see Totland 1997b for more details). The experiment was initiated

on 16 July 1994 when eleven 60 × 60 cm plot pairs were randomly chosen in a randomized block design within a 15 × 20 m area on a solifluction mat. One plot of each pair was randomly assigned to receive the OTC treatment, and the other was the control.

During all four seasons the flowering phenology of *R. acris* was measured by counting all open flowers inside the plots every 1–3 days. Flowers were considered open when the anthers were exposed to pollinators, and petals had their fresh yellow colour intact. Seed production of plants inside the plots was measured by collecting the seed head of the first open flower on three to ten randomly selected plants each year when seeds were ripe. Seed heads were stored in paper bags for at least 3 months at room temperature. Thereafter, the number of seeds and undeveloped achenes (include both unfertilized and aborted) were counted. Average seed weight per flower was measured by weighing all mature seed from a flower collectively on a Sartorius micro-balance to the nearest 0.01 mg, and dividing this weight by the number of seeds weighed. In 1996, the largest leaf was collected on each plants, whereas in 1997 I collected all basal leaves on the plants. Leaves were weighed after at least 3 months of air-drying at room temperature.

Phenotypic selection analysis

I collected data for the phenotypic selection analyses during the 1997 season. Plants in OTCs and control plots that had just entered anthesis (indicated by half-opened flowers and unopened anthers) were randomly selected during the flowering season, and the date of complete petal opening of each flower on the plants was noted. Plant size was estimated at the time of seed collection as described in the previous section. Seeds from all flowers on each plant were collected when mature, and total seed production of the selected plants was determined as described above.

Statistical analyses

All statistical analyses were done with SYSTAT 7.0.1. I used three-factor ANOVA to test the hypothesis that OTC treatment, year, and block affected growth and reproduction of *R. acris*. In these analyses treatment and year were considered fixed factors. Consequently, their *F*-ratios were calculated using the mean square of the error term in the denominator. To avoid pseudoreplication, I used means of each plot each year as the response for each variable. Seed:ovule ratio was arcsine-transformed to meet the assumptions of homogeneity of variance and normally distributed errors of ANOVA. No other variables required transformation.

To examine whether flowering start, peak, and end in the plots differed between OTCs and controls and between years, I used a multivariate ANOVA, with flowering start, peak and end (square-root-transformed Julian day numbers) as dependent variables, and treatment, year, their interaction, and block as predictor variables.

I used the number of flowers counted on the day with maximum flower density for each plot in each year as an estimate of plant density. Since the majority of plants produce only one flower, and since the number of flowers per plant does not differ significantly between OTC and control plots, this number will estimate reasonably well the density of reproducing plants in a given year and plot. To assess whether plant density responded to the treatment and changed during the study, I subjected these data to the same three-factor ANOVA model described above. The maximum number of flowers was square-root-transformed before analysis.

I used multivariate selection regression analysis (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b; Endler 1986) to estimate the magnitude and direction of selection acting on the quantitative plant traits measured (flowering time, average leaf weight (total leaf weight/leaf number), leaf number, flower number) in 1997. Separate analyses were done for control and OTC plants. I used relative total seed number (seed number/mean seed number) as the fitness estimate. Separate relative seed numbers were calcu-

lated for each treatment. Before analyses each quantitative trait was standardized (mean = 0, SD = 1) by subtracting the mean of the trait from each value and dividing by the SD. This was done separately for each treatment because of differences in means and SDs of the traits between treatments. Standardization of traits facilitates comparisons of selective forces among traits and between treatments. The standardized linear (directional) selection differentials were estimated as the covariance between relative fitness and each standardized trait, and their significance was assessed by Pearson correlation. Standardized selection differentials estimate the amount by which selection shifts the trait mean between actual and potential parents within a generation, and thus describe the total selection acting on a trait, including both direct selection and indirect selection acting on correlated traits. The standardized linear (directional) selection gradients were estimated as the vector of partial regression coefficients from multiple regressions of relative fitness on the set of the standardized traits measured. Standardized selection gradients describe the direct selection on a trait, after accounting for selection on the other traits measured. It is important to distinguish between phenotypic selection and the evolutionary response to selection. Selection acts on phenotypes, regardless of their genetic basis, and causes immediate effects within a generation on traits. On the other hand, evolutionary responses to selection, the genetic change from one generation to the next, depend on heritability of the traits (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b; Endler 1986). I used the homogeneity of slopes test (ANCOVA) to test whether the relationship between relative fitness and the measured traits differed between OTC and control plants. A significant treatment by trait interaction in an ANCOVA with relative fitness as the dependent variable would imply a significant difference in the relationship between fitness and the trait for control and OTC plants. Because of relatively small sample sizes (46 for control plants and 40 for OTC plants) I did not attempt to estimate non-linear selection differentials and gradients. I used the delete-one-jackknife procedure in SYSTAT 7.0.1 to examine the accuracy of the estimated selection gradients. Jackknifed estimates differed very little from the original estimates, and I therefore only presents the original estimates of selection gradients.

Results

Flowering phenology

Flowering start and peak of *R. acris* occurred more or less on the same dates in 1994–1996 (mid-July), whereas in 1997 flowering started and peaked *c.* 2 weeks earlier than in the three previous years (Fig. 1). In all years, except 1996, there were small differences in the flowering phenology inside OTCs and in control plots. Multivariate analysis of variance showed that flowering phenology differed significantly between years (Wilk's $\lambda = 0.05$, $F_{9,165} = 46.27$, $P < 0.00001$) and between OTCs and control plots (Wilk's $\lambda = 0.58$, $F_{3,68} = 16.68$, $P < 0.00001$), and also revealed a significant treatment by year interaction (Wilk's $\lambda = 0.71$, $F_{9,165} = 2.81$, $P = 0.004$).

There were large differences among years in the maximum number of open flowers (Fig. 2). In addition, while flowering was most abundant in OTC plots during the first treatment year, it was lower in warmed plots in all subsequent years. Analysis of variance showed that maximum flower number differed significantly among years ($F_{3,70} = 19.44$, $P < 0.00001$), but not between treatments ($F_{1,70} = 1.27$, $P = 0.26$). The drop in flower

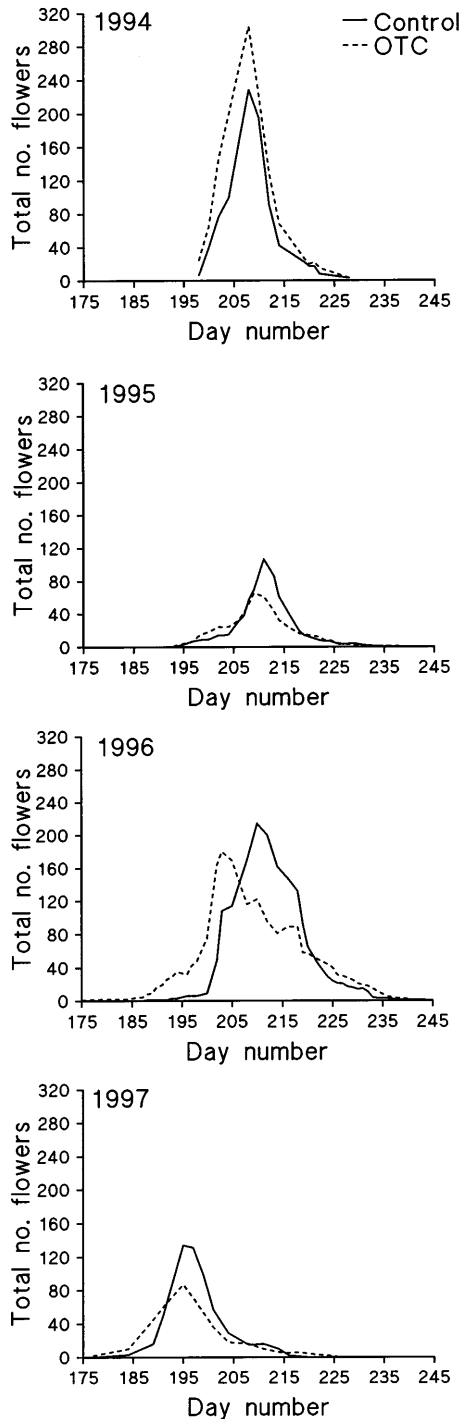


Fig. 1 Flowering phenology of *Ranunculus acris* at Finse in control and open-top-chamber (OTC) plots from 1994 to 1997. Day number 175 is 24 June

abundance in OTCs from above that in controls in 1994 to below that in controls in 1995 and subsequent years resulted in a close to significant treatment by year interaction ($F_{3,70} = 2.20$, $P = 0.095$). Flower abundance differed significantly between blocks ($F_{10,70} = 2.10$, $P = 0.036$).

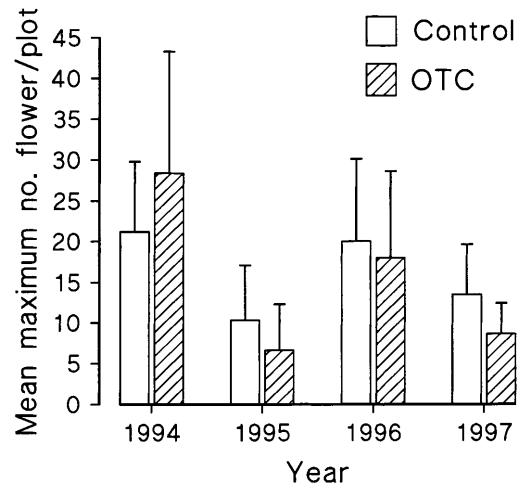


Fig. 2 Bar graph showing mean maximum number of flowers of *R. acris* per control and OTC plot at Finse from 1994 to 1997. Vertical lines are SEs

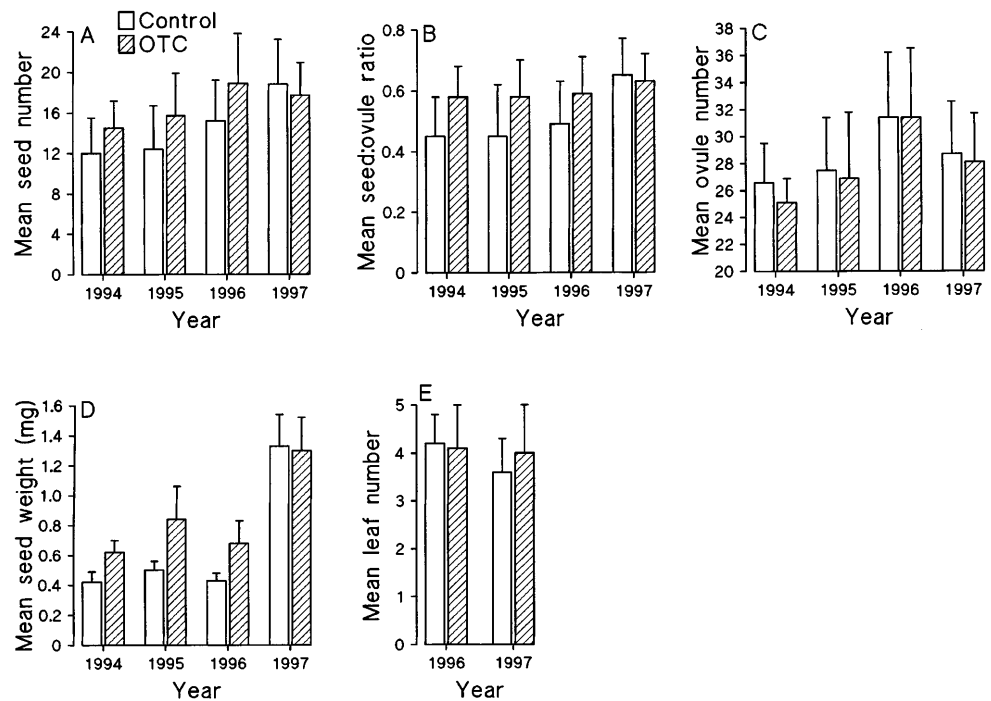
Reproduction and growth

Most of the measured variables of *R. acris* responded positively to the OTC treatment (Fig. 3), and responses were quite consistent in all years, except for the last year when all reproductive variables decreased inside OTCs (Fig. 3). Pooled across all years, seed number, seed ovule ratio, and seed weight increased by 13, 14, and 22% respectively, in response to the OTC treatment. Seed weight was substantially higher in the last year compared to previous years (Fig. 3). Analyses of variance showed that all variables, except ovule number and leaf number, responded significantly to the OTC treatment, and that leaf number (only measured during the two last years) was the only variable that did not differ between years (Table 1). The effect of the OTC treatment on seed weight differed significantly among years (significant treatment by year interaction, Table 1) because of the lack of a treatment effect in 1997. The block effect was significant on seed number and seed ovule ratio (Table 1). In 1997, 21.9 and 29.6% of the plants in the control and OTC plots, respectively, produced more than one flower. These proportions did not differ significantly ($\chi^2 = 1.27$, $df = 1$, $P = 0.26$). Of those plants producing three flowers, four were in OTCs and three in control plots. No plants produced more than three flowers. The average weight of the largest leaf on control plants in 1996 was 8.00 mg (SD = 1.86), whereas the average weight of such leaves in OTCs was 8.68 mg (SD = 2.47), a non-significant difference (Table 1). In 1997, average leaf weight of plants in control plots was 7.97 mg (SD = 2.08), whereas average leaf weight of plants in OTCs was 10.05 mg (SD = 1.55), a significant difference (Table 1).

Phenotypic selection analysis

Selection differentials and gradients on flower number of *R. acris* were significant in both control and OTC-plants

Fig. 3 Bar graphs showing **A** mean seed number, **B** mean seed:ovule ratio, **C** mean ovule number, **D** mean seed weight, and **E** mean leaf number of *R. acris* plants in control plots and OTCs at Finse from 1994 to 1997. Vertical lines are SDs. Reproductive data are based on the first open flower on each measured plant



(Table 2, Fig. 4), showing that selection acted in direction of increased flower number in both warm and natural conditions. The relationship between fitness and flower number was similar for control and warmed plants (homogeneity of slopes test; $F_{1,82} = 0.90$, $P = 0.35$). There was no evidence of selection acting on flowering time in the two groups (Table 2, Fig. 4). There was a close to significant direct selection favouring smaller leaf number in control plants, whereas no selection on this trait in warmed plants was evident (Table 2, Fig. 4). Homogeneity of slopes test showed a close to significant difference in the relationship between leaf number and fitness in the two groups ($F_{1,82} = 3.43$, $P = 0.068$). However, there was no indication of total selection (differential) acting on leaf number in any of the groups (Table 2). Selection favoured plants with large leaves in natural conditions, whereas no selective forces were detected on leaf size in warmed plants (Table 2, Fig. 4). The difference in selection on leaf size between control and OTC plants was highly significant (homogeneity of slopes test; $F_{1,82} = 12.18$, $P = 0.0008$).

Discussion

Environmental effects on growth, flowering and reproduction

The onset of flowering of alpine *R. acris* showed little response to the warming treatment. Similar treatment has accelerated flowering phenology in many other species in arctic and alpine habitats (e.g., Henry and Molau 1997), because warming speeds up physiological processes preceding flowering. *R. acris* reaches anthesis

very early in the season under natural conditions (Totland 1993). Therefore, current flowering times may occur so early that any amelioration of the prefloration environment will not enable plants to bloom even earlier because physiological processes, such as pollen production, ovule maturation, and petal development, can not be accelerated further. In 1996, however, flowering was significantly advanced by the OTC treatment. It is likely that this was caused by an unusually early snowmelt in that year, coupled with relatively low prefloration temperatures (author, personal observations).

Reproductive output of *R. acris* increased significantly during the first three years of experimental warming, showing that seed number and weight were limited by temperature conditions during this period. This result is consistent with previous studies (e.g., Henry and Molau 1997) that have used OTCs to examine temperature effects on reproduction, and is explained by many factors. First, higher temperatures speed up pollen tube growth, and promote fertilization and embryo formation processes (Corbet 1990). Second, higher temperatures may accelerate seed maturation, and thereby enable plants to produce both more and bigger seeds (Corbet 1990). This is probably particularly important in *R. acris* which has green photosynthetic achenes throughout seed development. In such cases higher temperatures can speed up photosynthesis, and thereby alleviate carbon costs of reproduction (Bazzaz et al. 1979; Galen et al. 1993). There are many reasons for the lack of a warming effect on reproductive output in the last season. First, the increased reproductive output of warmed plants during the first three seasons may have incurred reproductive costs (Stearns 1992) that prevented them from producing a comparatively

Table 1 ANOVA on effects of OTC-treatment, year, their interaction, and block on response variables of *Ranunculus acris* at Finse in 1994–1997 (1996–1997 for leaf number, 1996 and 1997 separately for leaf weight). Treatment and year are fixed effects.

Seed:ovule ratio is arcsine-transformed (*SS* sums of squares, *df* degrees of freedom, *MS* mean square, *P* significance level, *R*² coefficient of determination for significant models)

Dependent variable	Source of variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i> -ratio	<i>P</i>	<i>R</i> ²
Seed number	Treatment	95.66	1	95.66	6.88	0.01	0.46
	Year	370.61	3	123.54	8.89	0.00005	
	Treatment × Year	78.84	3	26.28	1.89	0.14	
	Block	298.41	10	29.84	2.15	0.03	
	Error	972.84	70	13.90			
Seed:ovule ratio	Treatment	0.16	1	0.16	10.37	0.002	0.44
	Year	0.26	3	0.09	5.53	0.002	
	Treatment × Year	0.10	3	0.03	2.07	0.11	
	Block	0.33	10	0.03	2.12	0.03	
	Error	1.08	70	0.02			
Ovule number	Treatment	9.95	1	9.95	0.65	0.42	0.36
	Year	364.46	3	121.46	7.97	0.0001	
	Treatment × Year	5.89	3	1.96	0.13	0.94	
	Block	215.95	10	21.59	1.42	0.19	
	Error	1106.81	70	15.24			
Seed weight	Treatment	0.79	1	0.79	34.95	<0.000001	0.87
	Year	0.19	3	3.06	135.17	<0.000001	
	Treatment × Year	0.40	3	0.13	5.81	0.001	
	Block	0.21	10	0.02	0.95	0.49	
	Error	1.59	70	0.02			
Leaf number	Treatment	0.06	1	0.06	0.10	0.74	
	Year	1.39	1	1.39	2.28	0.14	
	Treatment × Year	0.67	1	0.67	1.10	0.30	
	Block	7.59	10	0.76	1.25	0.30	
	Error	18.23	30	0.61			
Leaf weight (1996)	Treatment	2.56	1	2.56	0.38	0.55	
	Block	28.97	10	2.90	0.43	0.90	
	Error	66.68	10	6.67			
Leaf weight (1997)	Treatment	24.32	1	24.32	6.94	0.025	0.62
	Block	32.26	10	3.23	0.92	0.55	
	Error	32.05	10	3.51			

Table 2 Linear standardized selection differentials and linear standardized selection gradients for *R. acris* plants inside open-top chambers and in control plots at Finse in 1997, based on covariance (differentials) and linear multiple regression (gradients). The fitness estimate used was total seed number. Flowering date is the date

when the first flower entered anthesis. Leaf weight is the average weight of leaves. **Bold** differentials and gradients are significant at the 0.05 level. (*Values in parentheses* significance values, *n* sample size, *R*² is the coefficient of multiple determination, *F* is the *F*-ratio, and *P* is significance level from multiple regression)

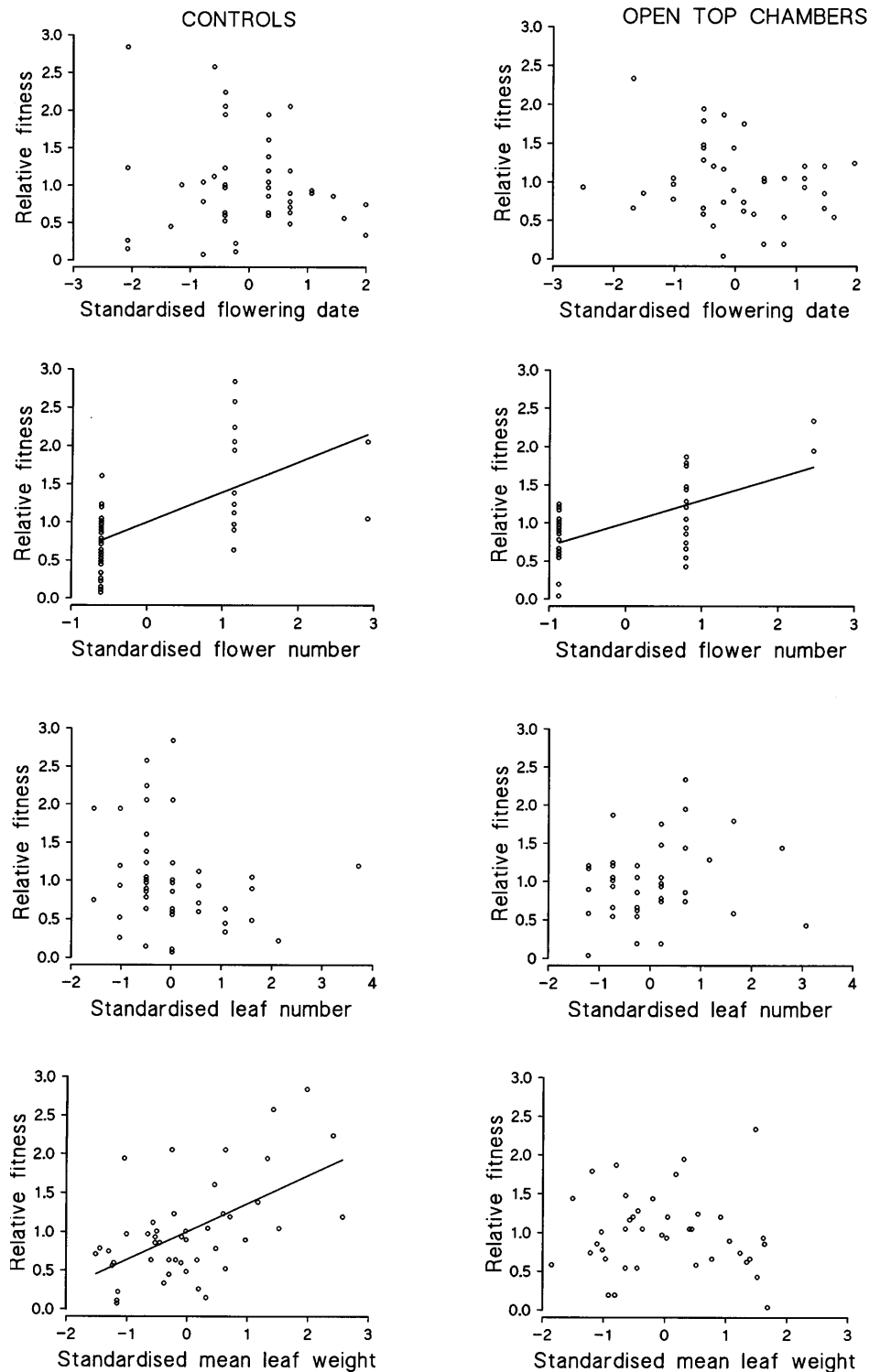
Trait	Control		Open-top chambers	
	Differential	Gradient	Differential	Gradient
Flowering time	−0.09 (0.38)	0.06 (0.42)	−0.10 (0.21)	0.03 (0.71)
Flower number	0.39 (<0.00001)	0.32 (0.00003)	0.30 (0.00004)	0.33 (0.0001)
Leaf number	−0.14 (0.14)	−0.13 (0.06)	0.09 (0.25)	−0.04 (0.57)
Leaf weight	0.36 (0.00004)	0.26 (0.0007)	−0.03 (0.67)	−0.05 (0.41)
<i>n</i>	46	46	40	40
<i>R</i> ²		0.57		0.39
<i>F</i>		13.84		5.48
<i>P</i>		<0.00001		0.002

large seed crop in the last season. Second, temperatures during the flowering and fruiting period in the last season were very high, and may have been so benign that seed number and weight of control plants were not constrained by temperature.

Warming had no effect on flower number in the 4th year of experimentation. In *R. acris*, and most other alpine and arctic species (Bliss 1971), flower-bud primordia are formed in late summer the year before flower-

ing. In another study on *R. acris* at Finse (Totland and Eide 1999), plants flowering late in a season had significantly lower ability to produce a flower in the subsequent year, compared to early-flowering plants, suggesting that resource demands between flower bud formation and seed maturation conflicted. It is likely that this conflict was particularly intense in plants inside OTCs, because of their higher seed number and seed weight, and that this prevented plants inside OTCs from producing more

Fig. 4 Relationships between relative fitness (total seed number/mean seed number) and standardized quantitative traits [(trait value – mean trait value)/standard deviation of trait values] in *R. acris* in control plots ($n = 46$, left) and open-top chambers ($n = 40$, right) at Finse in 1997. Regression lines are shown where there was a significant ($P < 0.05$) relationship between relative fitness and standardized traits in multiple regression



flowers than control plants, despite the higher temperatures inside OTCs. Totland and Birks (1996) found that average seed weight of *R. acris* populations at Finse decreased significantly along an altitude gradient, thus agreeing with the results obtained here.

Increased seed number and weight inside OTCs did not result in increased density of flowering plants of

R. acris in warmed plots. A likely reason for this is that although OTCs improved one important factor for seed germination, namely temperature, seed germination may have been negatively affected by the effects of increased temperature on other factors. For example, litter and soil moisture (Bliss et al. 1994), which probably are important for the germination success of *R. acris*, de-

crease inside OTCs (Marion et al. 1997), and this most likely reduced the germination success of *R. acris*. Other studies have shown that increased cover of grasses and shrubs, in particular, constrained or reduced population growth of herbs under warmed conditions, due to increased shading (e.g., Chapin et al. 1995). In the OTCs used here, the combined density of grasses and sedges was 14% higher inside OTCs than in control plots, whereas the density of *Salix herbacea* (by far the most abundant shrub) was actually 20% lower inside OTCs than in control plots (estimated with a point frame, Ø. Totland and W. Eide, unpublished work). In addition to a higher cover of graminoids, their shading effect is further increased by their taller growth inside OTCs (author, personal observations). Thus, it is likely that the higher grass cover inside OTCs reduced germination and establishment opportunities for *R. acris* due to increased shading and decreased availability of suitable germination sites (Bell and Bliss 1980). The density of flowering *R. acris* plants fluctuated greatly between years, showing that factors other than temperature are important for density. In particular, heavy disturbance from rodents of the ground and predation of flower primordia of *R. acris* during August and September in 1994 most likely caused the great decrease in flower density in 1995.

Average leaf weight in the last year of experimentation, but not leaf number, was substantially higher inside OTCs than in control plots. Increased growth in warmed conditions is common (Chapin et al. 1995; Henry and Molau 1997; Wookey and Robinson 1997), and show that the growth of *R. acris* and other alpine and arctic species is constrained by low temperature conditions. On the other hand, since grass cover was highest inside OTCs, the increased leaf growth of *R. acris* inside OTCs may also be a compensatory response to increased shading.

Environmental effects on phenotypic selection

The phenotypic-selection regression analysis showed selection in direction of larger average leaf weight (an estimate of leaf size) in control plants, whereas no selection on leaf weight was evident in warmed plants. The apparent lack of selection pressure on leaf weight inside OTCs is caused by at least three factors. First, the increased temperatures inside OTCs speeded up physiological processes, such as photosynthesis, and possibly seed production became more or less independent of leaf weight, given that only one or few flowers are produced, because physiological activity was maintained at a sufficiently high rate by any leaf size. Second, the production of relatively large leaves in warmed conditions may be disadvantageous when costs associated with the production of such large leaves are so big that it occurs at the expense of reproductive success (Stearns 1992). Third, the production of relatively large leaves inside OTCs may be a compensatory mechanism caused by increased shading by graminoids, resulting in a reduction in the potential seed number of large-leafed plants. Thus, the

impression that there is no selection on plant size in warmed plots may be misleading. It is possible that there is selection for compensatory leaf growth in shaded conditions, despite short-term reductions in reproductive success, because losses in fitness due to this plastic change are compensated by increases in fitness due to plastic changes in other traits, such as subsequent reproduction, longevity or survival (Stearns 1992). Selection in the direction of increased leaf size under natural environmental conditions agrees with many previous studies (Samson and Werk 1986; Shipley and Dion 1992). One important reason for a positive correlation between leaf weight and reproductive output is that bigger plants produce more flowers. This is also the case for *R. acris* at Finse; there is a significant positive correlation between mean leaf weight and flower number in control plants ($r_{\text{Pearson}} = 0.33$, $P = 0.025$), whereas no such correlation was evident in warmed plants ($r_{\text{Pearson}} = 0.06$, $P = 0.70$).

Previous studies on *R. acris* at the same study area found that seed production was highest in early flowering plants (Totland 1994b, 1997a), because temperature and precipitation conditions were most benign early in the season, and because pollinator activity decreased as the season progressed (Totland 1993). In 1997, temperature did not change in a systematic manner as the flowering and fruiting season of *R. acris* progressed (correlation between daily mean temperature and day number = 0.21, $t = 1.55$, $P = 0.13$), and this may explain the relaxed selection pressure on flowering time during 1997. Probably, temperatures experienced by late-flowering plants were so benign that their seed production was not constrained by low temperatures.

No phenotypic selection on leaf number of control or warmed plants was detected, although a selection gradient in direction of fewer leaves of control plants was close to significant. Thus, it appears that leaf weight is more important for fitness than leaf number in this population of *R. acris*.

There was strong selection in the direction of increased flower number in both control and warmed plants. Since the flowers of *R. acris* reach anthesis sequentially, this relationship is not caused by a greater attractiveness to pollinators of plants that produce many flowers, as has been found for other species (e.g., Lawrence 1993; Mitchell 1994). Instead, the result more likely reflects a simple relationship between flower and ovule number, where the latter is highly positively correlated with seed number (control: $r = 0.79$, $P < 0.00001$; OTC: $r = 0.62$, $P = 0.00002$).

The phenotypic selection analysis shows that the strength of selection acting on traits can differ between habitats or seasons, depending on temperature conditions. Because temperatures in the season examined were higher than normal, it is possible that differences between experimentally warmed and control plots in selection differentials and gradients on traits in *R. acris* would be bigger in cooler seasons, because constraints on growth and reproduction would be greater in such seasons.

Consequences for environmental change

Short-term studies like this one that manipulate abiotic environmental factors to assess future fates of alpine and arctic populations also cause a number of indirect effects on other factors, such as vegetation cover, which also will change in a warmer climate (e.g., Pacala and Hurtt 1993). Therefore, although seed number and weight increased during this experiment, it is premature to conclude that population density will increase or remain stable in the future, because simultaneous changes in important environmental factors other than temperature were not investigated. In this short-term study warming had no positive effect on the density of reproducing plants, suggesting that an amelioration of climate conditions under climate change may not be sufficient to compensate for the longer-term negative effects that denser and taller vegetation may have on the density of this species, through negative impact on seed germination and seedling establishment.

The phenotypic-selection regressions show that selection on leaf weight in *R. acris* is mediated by environmental conditions, and this study is therefore one of the first to show that selection pressures on important life-history traits may change under global warming. Adaptation to the new environmental conditions under climate change may be an important process by which populations can withstand changed environmental conditions. However, before we know the heritability of important life-history traits, such as leaf weight, we are not able to assess whether evolutionary adaptation will be fast enough to track the speed of the environmental changes (Geber and Dawson 1993).

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