

# Differential selection of growth rate-related traits in wild barley, *Hordeum spontaneum*, in contrasting greenhouse nutrient environments

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## Keywords:

fitness;  
*Hordeum*;  
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 leaf area ratio;  
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 relative growth rate;  
 specific leaf area;  
 unit leaf rate.

## Abstract

Across-species comparisons show that inherent variation in relative growth rate (RGR) and its underlying traits are correlated with habitat productivity. In this study, we test the hypothesis that growth rate-related traits confer differential selective effects in contrasting nutrient environments. We specifically test whether high RGR is targeted by selection in nutrient-rich environments whereas low values of traits that underlie RGR [specific leaf area (SLA), leaf mass fraction and leaf area ratio (LAR)] confer a direct fitness advantage in nutrient-poor environments, resulting in selection of low RGR as a correlated response. We measured RGR, its underlying component traits, and estimated fitness in a range of wild barley (*Hordeum spontaneum*) accessions grown under high and low nutrient conditions. Selection on component traits differed between the two environments, while total selection of RGR was not significant. Using multiple regression and path analysis to estimate direct fitness effects, a selective advantage of high LAR and SLA was demonstrated only under nutrient-rich conditions. While supporting the view that observed associations between habitat richness and some RGR-component traits reflect adaptation to differing nutrient regimes, our data suggest that direct selection targets component traits rather than RGR itself.

## Introduction

The growth capacity of plant species, measured as the relative growth rate (RGR, increase in biomass per unit mass and time) under favourable experimental conditions, is correlated with habitat productivity: species from productive environments tend to have higher potential growth rates than species from poor environments (Grime & Hunt, 1975; Chapin, 1980; Lambers & Poorter, 1992). This association between potential growth and habitat productivity may or may not have an adaptive explanation, and different hypotheses have been proposed to account for the observed correlation. High RGR may be selected in productive habitats because it enables rapid

occupation of available space and thus promotes competitive ability in the dense vegetation stands that develop in these environments (Grime, 1979). A direct selective advantage of low RGR in unproductive environments is more difficult to envision, although it has been suggested by several authors (Grime & Hunt, 1975; Chapin, 1980). Alternatively, RGR-variation itself may not be adaptive, but selection targeting correlated characters may differ between habitats that vary in productivity, resulting in differential indirect selection of RGR (Lambers & Dijkstra, 1987; Poorter, 1989; Lambers & Poorter, 1992). Some support for the latter hypothesis comes from observations that slow-growing species often show high tissue density, high investment in cell wall materials and defence compounds, and low specific leaf area (SLA, leaf area per unit leaf mass) (Poorter & Remkes, 1990; Niemann *et al.*, 1992; Schläpfer & Ryser, 1996; Cunningham *et al.*, 1999; Ryser & Wahl, 2001). Such traits are associated with increased tissue persistence (Reich, 1993; Ryser, 1996), which is believed to be particularly important in

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nutrient-poor habitats because of the high costs of tissue replacement, but they also negatively affect growth rate (Lambers & Poorter, 1992). This suggests that there may be a trade-off between high growth capacity and tissue persistence that leads to different adaptive outcomes in habitats of contrasting productivity: in productive habitats rapid growth itself may be favoured, but in unproductive environments slow growth may be selected indirectly via selection on correlated traits that increase tissue persistence (Aerts & Berendse, 1989; Lambers & Poorter, 1992). In addition, nutrient limitation has been hypothesized to select for high root biomass allocation, and consequently low leaf mass fraction (LMF, biomass allocated to leaves), which also reduces the capacity for rapid growth (Lambers & Poorter, 1992). Consistent with the trade-off hypothesis, species from productive environments may still grow faster than species from unproductive environments when tested under nutrient-limited experimental conditions (Poorter *et al.*, 1995).

Direct empirical evidence for the adaptive significance of natural variation in growth rate-related traits is scarce, and the above hypothesis mainly rests on across-species correlations between inherent growth characteristics and habitat characteristics. Fitness effects of variation in growth rate-related traits have been further explored by assessing their impact on competitive ability in interspecific competition experiments. In such studies, RGR is often decomposed into its underlying component traits: SLA, LMF, leaf area ratio (LAR, ratio of total leaf area and plant biomass), and unit leaf rate (ULR, plant biomass increase expressed per unit leaf area); by definition,  $RGR = ULR \times LAR = ULR \times SLA \times LMF$  (Hunt, 1978). These studies have generally found no effect of RGR, and inconsistent effects of the underlying components SLA and LAR, which were found to be positively related (Rösch *et al.*, 1997), negatively related (Roush & Radosevich, 1985), or not related (Walck *et al.*, 1999) to competitive ability.

Selection pressures on growth traits could be estimated more directly in intraspecific studies from the covariance between these traits and fitness. This requires information on growth and fitness of individual plants, or, alternatively, on breeding values or family means of genotypes that show heritable variation in growth traits (Mauricio & Mojonner, 1997). Despite the potential of this method to demonstrate differential selective effects of growth traits in contrasting environments, such studies are rare. Making use of naturally occurring variation, the phenotypic selection approach has been used to explore nutrient-dependent fitness consequences of allocation patterns (Cheplick, 2001), RGR (Kik *et al.*, 1991) and growth rate-components (Biere, 1996). Differential fitness effects in contrasting nutrient environments were demonstrated for SLA and LMF in the perennial hay-meadow species *Lychnis flos-cuculi* (although effects were different in natural fields and under experimental greenhouse conditions; Biere, 1996)

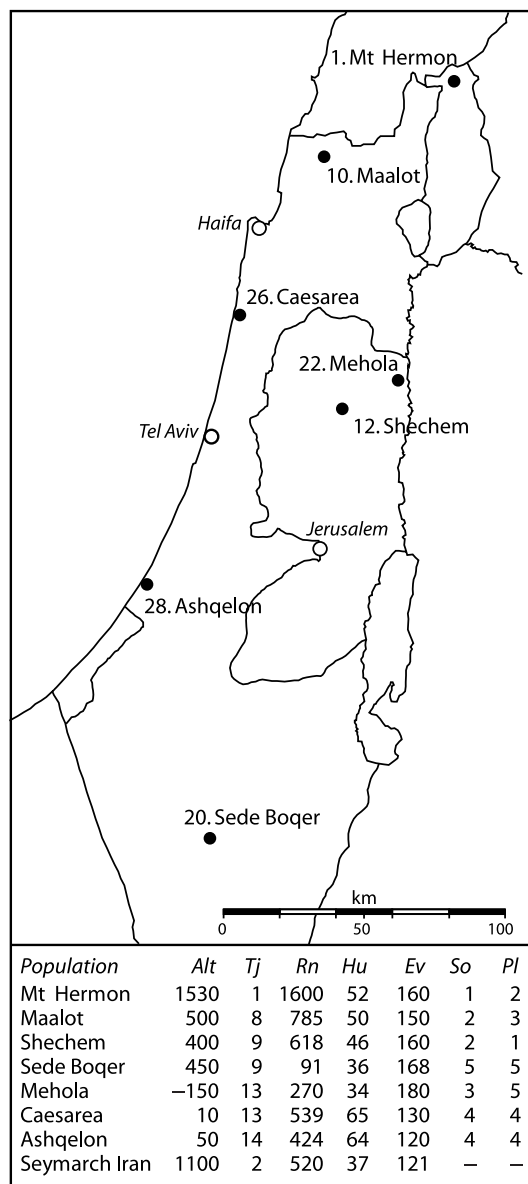
and for root biomass allocation in the annual *Amaranthus albus* (Cheplick, 2001). In both studies, directional selection was observed under nutrient-rich greenhouse conditions, but no fitness consequences could be demonstrated under nutrient-poor greenhouse conditions, challenging the view that low values of SLA and LMF are selected for under low nutrient supply.

Given the paucity of empirical evidence, and despite extensive speculation, the adaptive significance of inherent variation in RGR-components as observed within and among natural populations remains largely to be demonstrated. Here we report on the selective effects of variation in RGR and its component traits in wild barley (*Hordeum spontaneum* Koch) in contrasting nutrient environments. *Hordeum spontaneum*, the wild progenitor of cultivated barley, is a self-pollinating annual grass that occurs in a wide range of habitats across the eastern Mediterranean basin and western Asia (Nevo *et al.*, 1984; Nevo, 1992). We use selection analysis, linking growth traits to seed production in a range of *H. spontaneum* accessions at both high and low nutrient supply, to address three main questions: (1) Which components of the RGR-complex are under selection? (2) Is RGR under direct selection or is there only indirect selection via underlying components? And (3) how does selection vary between contrasting nutrient environments? Specifically, we will test the hypotheses that high RGR is targeted by selection in nutrient-rich environments whereas low values of SLA, LMF and LAR confer a direct fitness advantage in nutrient-poor environments, resulting in selection of low RGR as a correlated response. Furthermore, we hypothesize that leaf tissue density (LTD) underlies differential selective effects of SLA in the contrasting nutrient environments. The significance of growth traits for plant fitness is explored in relation to other plant characteristics that affect plant size and fitness, viz. initial seed size and preanthesis growth duration.

## Material and methods

### Plant material

We studied a total of 36 wild barley accessions (i.e. descendants from a single plant collected in the field and propagated by selfing) from nine populations in Israel and Iran, that were chosen to represent maximal variability in geographical and environmental conditions (Fig. 1; four accessions used per population). Sampling of these populations was described in Nevo *et al.* (1979, 1986), and all accessions were propagated for four to six generations under common garden and greenhouse conditions prior to the experiment. As *H. spontaneum* is a self-fertilizing species with very low natural outcrossing rates (Brown *et al.*, 1978), accessions were assumed to be genetically nearly homogeneous lines. Accessions from these populations show significant inherent differences in growth characteristics (van Rijn *et al.*, 2000).



**Fig. 1** Sampling locations in Israel and environmental data of the *Hordeum spontaneum* populations. For each population, the following characteristics are given: altitude (alt, in m); mean January temperature (Tj, in °C); annual precipitation (Rn, in mm); mean midday humidity (Hu); mean annual evaporation (Ev); Soil type (So: 1. Terra Rossa; 2. Rendzina; 3. Alluvium; 4. Sandy loam; 5. Loess); Plant community (Pl: 1. Batha; 2. Marginal batha; 3. Liveoak maquis; 4. *Ceratonia Pistacia* park forest; 5. Steppic). Environmental data and population codes are from Nevo *et al.* (1984, 1986).

### Experimental design

The experiment followed a randomized complete block design with four blocks, nine populations, four accessions per population, and two nutrient treatments. Within each block, five randomly positioned plants per

accession were grown at each nutrient level (1440 plants in total). Of these five plants, two were used for growth analysis with destructive harvesting during the early phase of maximum growth. One plant was harvested at the onset of flowering to determine plant size and leaf area at the end of the vegetative growth phase, and the two remaining plants were allowed to complete their reproductive phase and their seed production was estimated.

### Growth conditions

Seeds were stored at 40 °C for 2 weeks in order to break dormancy (Gutterman *et al.*, 1996) and caryopses were cleaned from awns, lemmas, paleas and glumes. On four consecutive days, corresponding to the four blocks in the design, 20 caryopses per accession were germinated in 85 mm petri dishes on filter paper moistened with demi water (day 0). After a short vernalization period of 7 days at 4 °C (light/dark: 10/14 h, PAR 3–12  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), petri dishes with germinated seeds were transferred to a growth cabinet (light/dark: 16/8 h, 22/16 °C, PAR 150–180  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). On day 9, seedlings were transplanted individually into 1000 mL pots containing 1400 g fire-dried quartz sand (fraction 0.2–0.6 mm; Eurogrit, Nieuwegein, The Netherlands), and were placed on greenhouse benches. Greenhouse temperature was maintained at  $21 \pm 2$  °C during the day and  $16 \pm 1$  °C at night. Additional lighting was provided by HPL-T 400 W lamps (Philips Nederland BV, Eindhoven, The Netherlands), ensuring a minimum light intensity at plant level of PAR 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (maximum approximately 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on sunny days) and a constant 16 h day length during the entire experiment.

Plants were supplied with either 1/2-strength or 1/32-strength modified Hoagland nutrient solution ('high' vs. 'low' nutrient treatment). Full-strength solution contained 5 mM  $\text{Ca}(\text{NO}_3)_2$ , 5 mM  $\text{KNO}_3$ , 1 mM  $\text{KH}_2\text{PO}_4$ , 2 mM  $\text{MgSO}_4$ , 92.5  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 18.3  $\mu\text{M}$   $\text{MnCl}_2$ , 1.5  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.6  $\mu\text{M}$   $\text{CuSO}_4$ , 1.0  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , and 174.4  $\mu\text{M}$   $\text{FeEDTA}$ . The high and low macro-nutrient concentrations were derived from a full-strength solution by dilution with demi water. At both levels the concentration of micro-nutrients (the latter six compounds) was held at 1/2-strength. During vegetative growth, pots were placed on saucers to avoid nutrient leaching. Within each nutrient level all plants received the same amounts of nutrient solution, but the amounts varied between the two nutrient levels and also throughout the growing season to accommodate for the larger water requirement of bigger plants. To check whether nutrients accumulated in the pots because of prolonged watering with Hoagland solution, 10 randomly chosen pots were flushed with 1 L demi water after 4 months and  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , P and K-concentrations were determined spectrometrically in the leaching solution. Accumulation of available N, P and K appeared to be minimal, and the amounts of nutrients

flushed from the pots were <50% of the quantities given with each Hoagland administration.

### Data collection

Prior to germination, 80 dried and cleaned caryopses per accession were weighed in groups of 10. For growth analysis, plants were harvested on day 17 and 26 after the onset of germination. At these harvests, one plant per accession (per treatment) was removed from each block in the experiment and was separated into roots, stems (including leaf sheaths), green leaf blades, and dead leaf parts (when present). We measured fresh weights of leaf blades, and total green leaf area was determined with a digital image analysis system (Delta-T Devices Ltd., Burwell, Cambridge, UK). Roots, leaves and stems were oven-dried at 70 °C for at least 48 h and dry weights were determined.

At the onset of flowering, which was defined as the first day that the flag leaf was fully expanded and the awn tips had emerged from the developing ear, one plant per accession (per treatment) was harvested from each block. The moment of this harvest differed between individual plants. Dry weights of roots, shoots, green leaves and dead leaf parts were determined as described above, and total green leaf area was estimated by measuring leaf area of a random sample of leaves and calculating total green leaf area based on the dry weight ratio of measured and unmeasured leaves.

Seed production was measured on the two remaining individuals per accession and per nutrient level in each block. In *H. spontaneum*, seed dispersal units shatter from the ears upon ripening. Therefore, reproductive plants were checked at least once per week to manually collect developed units before they were shed from the plant. Each successful unit is composed of one fertile and two sterile spikelets, and contains one kernel. Collected dispersal units were dried at 40 °C for at least 6 weeks, counted, checked for the presence of a kernel to distinguish aborted seeds from fertile seeds, and bulk-weighed per plant. Total seed production was estimated as the total weight of all dispersal units that contained fertile seed. After plants had died the number of finished reproductive tillers was counted, equalling the number of ears produced. For practical reasons, plants were removed from the experiment if they had not completely senesced naturally 140 days after the onset of flowering. At this time, some plants still showed maturing tillers but initiation of new tillers was minimal. In these plants, the total seed production score was adjusted to include seeds from still developing ears by assuming mean values for the number of fertile seeds per ear and seed weight, as calculated over the first 140 flowering days in each individual.

Flowering time, defined as the number of days from transfer of seedlings to the greenhouse until the onset of flowering, was recorded for the three plants per accession

and treatment in each block that were not included in the growth analysis.

### Data analysis

Based on the data collected during the growth analysis harvests, RGR and ULR of plants from each accession were derived according to Hunt (1978) by pairing of plants from the same block and nutrient level across the two harvests. LAR, SLA, LMF and LTD (estimated as the ratio of leaf dry weight and leaf fresh weight) were calculated at each harvest and the values for two individuals (in the same block and nutrient level) were averaged across the harvests to yield a mean score for the harvest interval. Total plant dry weight, LMF and RMF (root mass fraction) at the onset of flowering were determined based on living tissue, i.e. excluding dead leaf parts.

Among-genotype variance components for growth, flowering and fitness traits were calculated for the two nutrient environments separately, using variance components analysis (SAS, procedure VARCOMP, REML estimation). After removing block variance, the among-genotype variance was estimated as the proportion of variance explained by differences among populations plus differences among accessions nested within populations. These estimates include possible maternal effects, and therefore tend to be overestimates of the genetic component of variance (Falconer & Mackay, 1996).

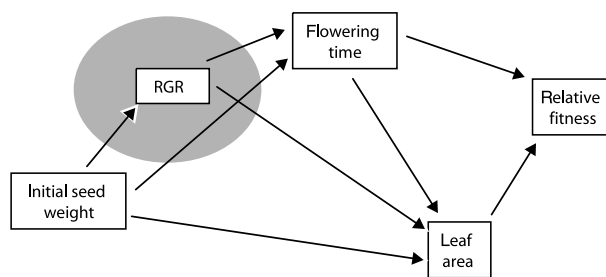
We used analysis of variance to assess the nutrient, population and accession effects on growth, flowering and fitness traits (SAS, procedure GLM, type III EMS). In these analyses, we considered the variables nutrient and population as fixed factors, whereas block and accession were treated as random factors. Accession was nested within population. Factorial models were fitted in which all three-way interaction terms with block were pooled in the error term.

Following Lande & Arnold (1983), we measured selection on individual traits via the covariance between traits and fitness using regression analyses. Selection was quantified using three different models: univariate regression, multivariate (multiple) regression, and a path analysis extension of multivariate regression via structural equation modelling. Selection analyses were performed separately for the two nutrient environments, and were based on accession means (cf. Mauricio & Mojonner, 1997). In order to compare the strength of selection between traits and environments, relative fitness values were regressed on standardized phenotypic traits. Within environments, a relative fitness score was obtained by dividing the accession mean absolute fitness by the mean fitness of all accessions in that environment. All other traits were standardized to mean = 0 and SD = 1. Among the accessions, a significant trade-off was observed between the number and mean weight of

seeds produced (high-nutrients:  $r = -0.70$ ,  $P < 0.001$ ; low-nutrients:  $r = -0.61$ ,  $P < 0.001$ ; correlation of accession mean values,  $n = 32$ ). This trade-off is known to have resulted in different adaptive outcomes in *H. spontaneum* populations from different areas. Populations from mesic environments produce big (but few) seeds because this increases seedling establishment and competitive ability, while populations from xeric environments produce many (but small) seeds that confer a bet-hedging advantage in the unpredictable environments (Volis *et al.*, 2002b,c and references therein). In order not to bias our selection analysis in favour of traits associated with populations from xeric environments, we dealt with this inherent trade-off by using total seed biomass produced per plant as the lifetime fitness estimate, and not total seed number.

In univariate and multivariate linear regression, the (partial) regression coefficients  $\beta$  measure the directional selection gradients. Univariate linear regression of fitness on a phenotypic trait measures *total* directional selection on that trait, which incorporates indirect selection via effects of correlated characters. Multiple linear regression (MLR), including all focal traits in the analysis, quantifies *direct* directional selection on each trait after statistically controlling for variation in all other traits. In the multiple regression analysis, a potential problem of collinearity among the RGR-components was avoided by fitting three different models in which RGR was subsequently substituted by its constituent components: model 1 included RGR; model 2 included ULR and LAR; and model 3 included ULR, SLA and LMF. All regression coefficients were obtained using the REG procedure in SAS.

Multiple linear regression is commonly used in phenotypic selection studies, but this model rests on the assumption that the independent traits are hierarchically unstructured and all have a direct effect on fitness. If a structure of causality among the focal traits is known, direct selection of individual traits may be more accurately estimated by path analysis following an *a priori* defined path diagram (Kingsolver & Schemske, 1991). As a complement to the MLR analysis, we therefore restructured regression models into path diagrams and calculated direct selection as the total forward effect (i.e. the causal effect) of a trait on fitness, summing all forward paths that lead from the trait to fitness directly or via intermediate traits (structural equation modelling in SAS procedure CALIS, ML estimation). The path diagram used is shown in Fig. 2. All growth traits were assumed to have only indirect effects on fitness, either via the total green leaf area at the moment of flowering, which has a well-established relationship with seed production (Maddox & Antonovics, 1983; Arntz *et al.*, 1998), or via flowering time, which was highly correlated with total plant biomass at the moment of flowering (high nutrients:  $r = 0.92$ ; low nutrients:  $r = 0.84$ ).



**Fig. 2** Path diagram used in structural equation modelling. The shaded area represents the complex of relative growth rate (RGR)-related traits; in interchangeable models, RGR can be partitioned into its constituent components leaf area ratio and unit leaf rate or unit leaf rate, specific leaf area and leaf mass fraction (see also Fig. 4; in these models, all RGR-related traits potentially influence fitness indirectly via flowering time and leaf area at first flowering). All endogenous variables, i.e. with incoming arrows, were modelled with an error variable to absorb unexplained variance.

## Results

One population, from Mount Meron, performed poorly under the experimental conditions. Most plants failed to flower, possibly due to inadequate vernalization treatment during germination (Ellis *et al.*, 1988), and the population was removed from the experiment. All analyses are therefore based on 32 accessions from eight populations.

### Genetic trait variation

Within nutrient environments, a highly significant part of the observed variation in most traits was because of differences between populations or between accessions within populations (Table 1). Only for ULR (both nutrient levels) and RGR (low nutrients) no significant heritable variation could be demonstrated. Among-genotype variance proportions for other RGR-related traits ranged from 19.9 to 51.6%. For most traits, coefficients of variation as well as among-genotype variance proportions were of comparable magnitude in the high and low nutrient environments.

Lowering the nutrient supply caused a significant reduction in most of the growth, flowering and fitness traits measured (Tables 2–4; see also Table 1). Only ULR and flowering time remained unaffected, whereas LTD, root biomass allocation and mean seed weight increased. Although the vast majority of phenotypic variation was caused by the nutrient treatment, the overall analysis of variance revealed significant population and/or accession effects for most traits. In addition, genetic differences in phenotypic plasticity were suggested for leaf morphological traits (LTD, SLA and LAR), flowering time, plant biomass and leaf area at the onset of flowering, and all fitness traits, as indicated by significant nutrient  $\times$  population and/or nutrient  $\times$  accession interaction terms.

**Table 1** Mean values, units and coefficients of variation (CV) of early growth, flowering and fitness traits. Among-genotype variance components ( $\sigma_{\text{genotype}}^2$ ) were estimated as the percentage of total phenotypic variance explained by differences among populations plus accessions within populations after removing block variance, and include possible maternal effects.

Traits (units)	Mean		CV (%)		$\sigma_{\text{genotype}}^2$ (%)*	
	High	Low	High	Low	High	Low
Initial 10 kernel weight (mg)†	301		28.1		<b>75.4***</b>	
Early growth traits						
RGR (mg g <sup>-1</sup> day <sup>-1</sup> )	232	166	12.5	18.1	<b>20.9**</b>	15.5 <sup>ns</sup>
ULR (g m <sup>-2</sup> day <sup>-1</sup> )	9.2	8.7	14.6	20.4	0.7 <sup>ns</sup>	9.2 <sup>ns</sup>
LAR (m <sup>2</sup> kg <sup>-1</sup> )	26.6	20.3	7.5	10.5	<b>30.9***</b>	<b>19.9***</b>
SLA (m <sup>2</sup> kg <sup>-1</sup> )	49.6	45.5	7.5	10.9	<b>36.7**</b>	<b>33.6**</b>
LMF (g g <sup>-1</sup> )	0.54	0.45	5.3	7.6	<b>41.2***</b>	<b>51.6***</b>
LTD	0.11	0.14	7.5	9.8	<b>29.9**</b>	<b>26.0*</b>
Plant status at flowering						
Flowering time (days)‡	91	96	41.4	35.7	<b>77.9***</b>	<b>82.8***</b>
Total biomass (g)‡	18.7	2.5	41.7	53.6	<b>76.3***</b>	<b>72.6***</b>
LMF (g g <sup>-1</sup> )‡	0.32	0.16	21.5	40.2	<b>33.4**</b>	<b>30.2*</b>
RMF (g g <sup>-1</sup> )‡	0.26	0.49	22.0	23.0	<b>29.1***</b>	<b>47.2***</b>
Leaf area (cm <sup>2</sup> )‡	1734	83.8	35.5	51.8	<b>61.0***</b>	<b>31.9**</b>
Fitness components						
Tillers§	49.0	3.9	35.1	51.3	<b>20.3***</b>	<b>26.5***</b>
Total dispersal units‡	446	32.9	54.8	50.1	<b>56.4***</b>	<b>36.0***</b>
Fertile seeds‡	301	29.3	59.1	51.0	<b>46.4***</b>	<b>38.4***</b>
Mean seed weight (mg)	40.2	46.8	33.7	34.1	<b>89.1***</b>	<b>77.7***</b>
Total seed biomass (g)‡	10.9	1.3	44.5	46.8	<b>27.6***</b>	<b>32.9***</b>

\*Significance of  $\sigma_{\text{genotype}}^2$ , as determined by GLM analyses (type III EMS), denote most significant effect of either populations or accessions within populations; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Bold values are significant after sequential Bonferroni adjustment within nutrient levels (Rice, 1989).

†Initial 10-seed weight was determined before the start of the nutrient treatment.

‡Variable is log-transformed for variance component analysis.

§Variable is square root-transformed for variance component analysis.

RGR, relative growth rate; ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; LTD, leaf tissue density.

**Table 2** *F*-values from analysis of variance for early growth traits. For each trait, a separate factorial model was fit with all three-way interactions with block pooled in the error term.

	d.f.	RGR	ULR	LAR	SLA	LMF	LTD
Nutrient	1	126.9**	1.9	961.4***	27.5*	295.8***	179.1***
Population	7	2.8*	1.7	10.3**	4.9**	18.8***	1.2
Accession(P)	24	3.1	2.2	0.7	2.0	1.1	1.3
N × P	7	1.9	2.3	3.6**	2.4*	1.9	3.1*
N × A(P)	24	0.8	0.7	0.8	1.1	1.5	1.3
Block	3	3.7	1.7	9.4***	3.4	0.6	1.2
B × N	3	3.0*	3.2*	0.2	3.6*	3.0*	3.7*
B × P	21	1.1	0.6	0.8	1.0	0.6	1.8*
B × A(P)	72	0.7	0.9	1.3	1.3	1.3	1.0
d.f. (error)		91	91	93	93	93	93

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Population (P) and nutrient (N) are considered fixed factors, whereas accession (A) and block (B) are random factors. Accessions are nested within populations.

RGR, relative growth rate; ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; LTD, leaf tissue density.

Variation in RGR was governed strongly by both its component traits LAR and ULR, of which ULR appeared

**Table 3** *F*-values from analysis of variance for traits determined at the onset of flowering. See Table 2 for explanation. All traits are log-transformed to improve normality and/or homogeneity of error variance.

	d.f.	Flowering time	Total biomass	LMF	RMF	Leaf area
Nutrient	1	4.4	1523***	144***	246***	2130***
Population	7	4.3**	2.4*	4.1**	11.3***	1.1
Accession	24	11.8***	6.9***	1.2	1.4	3.2**
N × P	7	6.7***	3.3*	1.3	1.4	2.7*
N × A(P)	24	4.0***	1.7	1.6	1.0	1.7*
Block	3	1.1	5.5	0.6	0.8	1.9
B × N	3	1.6	0.7	1.8	1.9	1.3
B × P	21	0.4	0.9	1.0	0.9	1.6
B × A(P)	72	1.0	0.6	1.2	1.1	1.1
d.f. (error)		581	62	62	62	71

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

LMF, leaf mass fraction; RMF, root mass fraction.

most closely associated with RGR (Fig. 3). Direct effects of SLA and LMF on LAR were both strong and highly significant. The relationships between variables were of similar magnitude in the two nutrient environments, but

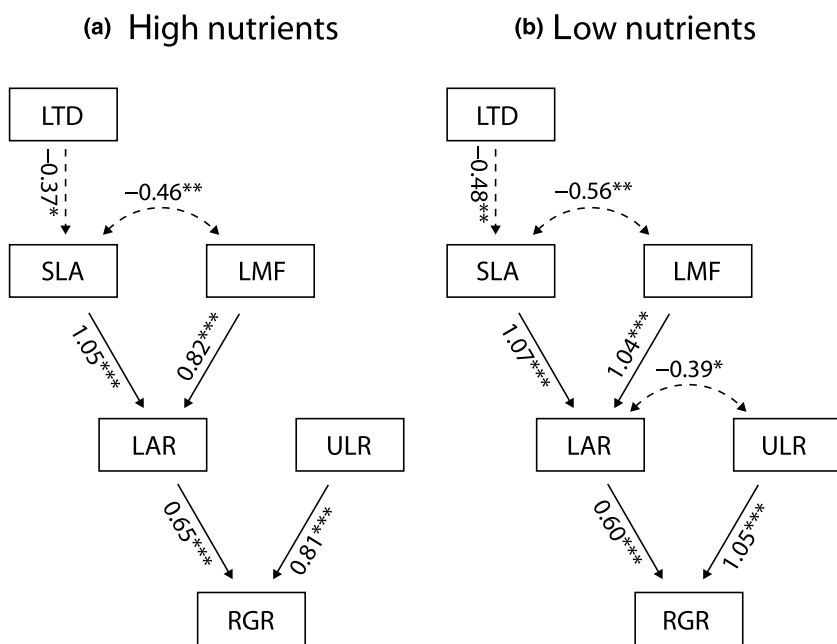
	d.f.	Tillers‡	Dispersal units†	Fertile seeds†	Mean seed weight	Total seed biomass†
Nutrient	1	2743***	1666***	1181***	37.7***	1202***
Population	7	1.3	7.0***	9.4***	16.4***	1.9
Accession	24	1.8	1.5	1.0	6.4***	1.8
N × P	7	2.0	2.1	1.7	2.8**	1.2
N × A(P)	24	1.9**	3.2***	3.2***	1.4	2.6***
Block	3	0.6	0.5	1.7	0.5	1.7
B × N	3	0.5	0.9	0.4	1.7	0.4
B × P	21	1.3	1.3	1.0	1.9**	0.8
B × A(P)	72	0.8	0.8	1.0	0.8	1.1
d.f. (error)		325	331	331	331	331

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

†Variable is log-transformed to improve normality and/or homogeneity of error variance.

‡Variable is square root-transformed to improve normality and/or homogeneity of error variance.

**Table 4** *F*-values from analysis of variance for fitness traits. See Table 2 for explanation.



**Fig. 3** Diagrams showing strength of relationships among the relative growth rate components in high (a) and low (b) nutrient environments. Double-headed arrows indicate Pearson correlation coefficients, and single-headed arrows indicate standardized partial regression coefficients. Analyses are based on line means for 32 accessions; only significant relations are shown (\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001).

a negative correlation between LAR and ULR was observed only under limiting nutrient conditions. This indicates that plants that maximize their total leaf area do this at the cost of biomass assimilation efficiency per unit leaf area when nutrients are limiting, but at high nutrient supply no such trade-off exists.

**Selection analysis**

Univariate linear regression showed different selection regimes in the two nutrient environments. At high nutrient supply, plants with high LAR, high SLA and low LTD were favoured by selection, whereas plants with big seeds and delayed flowering were more successful at

limiting nutrient supply (Table 5). Taking into account correlations among traits (see Table 6), MLR revealed that not all of these traits were directly selected for. In the nutrient-rich environment, both SLA and LMF had positive direct effects on fitness, whereas the direct effect of LTD was not significant (Table 7). Selection of genotypes with low LTD and selective neutrality of LMF, as observed in univariate regression, appeared to be caused by indirect effects via selection on SLA, as both LTD and LMF were negatively correlated with this trait. In the nutrient-poor environment, only flowering time had a significant direct effect on fitness; later onset of flowering resulted in higher seed output. Selection of big-seed genotypes, as suggested by a marginally significant

**Table 5** Univariate selection analysis of early growth traits, initial seed weight and flowering time. Linear regression coefficients  $\beta$  measure total directional selection on a trait, including indirect effects via correlated characters.

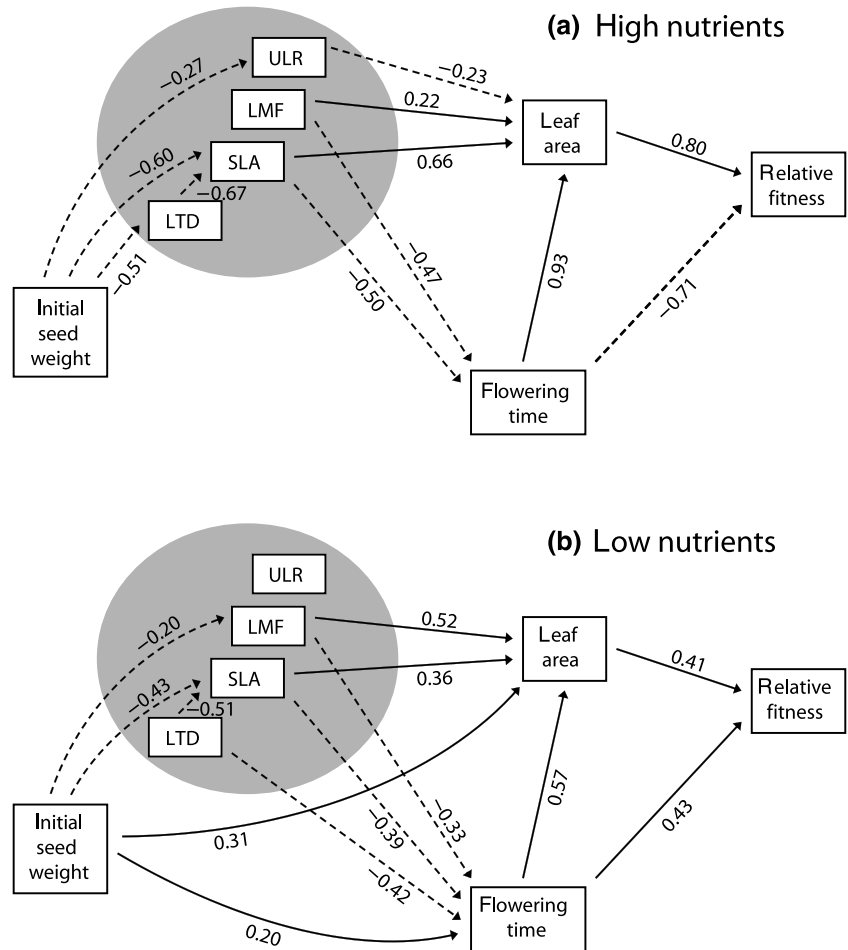
	High nutrients $\beta$	Low nutrients $\beta$
Initial seed weight	0.04	0.13*
RGR	0.05	0.06
ULR	-0.10	0.10
LAR	<b>0.17***</b>	-0.08
SLA	<b>0.14**</b>	-0.08
LMF	0.04	-0.01
LTD	<b>-0.16**</b>	-0.04
Flowering time	-0.06	<b>0.19***</b>

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Bold values are significant after sequential Bonferroni adjustment within nutrient levels (Rice, 1989).

RGR, relative growth rate; ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; LTD, leaf tissue density.

selection coefficient in univariate analysis, could be partly explained by a positive correlation between seed size and flowering time (i.e. plants from big seeds flowered later).

Taking into account the presumed causal relationships between the focal traits, we could confirm the direct fitness effect of SLA (i.e. the sum of the forward paths from SLA to relative fitness in the path diagram) at high nutrient supply, but the direct LMF-effect was found to be rather weak (Table 7). SLA and LMF had comparable effects on flowering time, but SLA was considerably more important than LMF in controlling total leaf area at anthesis, and thus fitness (Fig. 4a). At low nutrient supply, the direction of direct SLA and LMF effects on leaf area at anthesis was similar to their direction in the high-nutrient environment (high SLA and high LMF resulted in a large leaf area) but the relative importance of SLA and LMF in controlling leaf area at anthesis changed. Modelling LTD as a component trait of SLA in path analysis suggested that direct selection of this trait may be stronger than estimated by MLR (see Table 7). In either model, however, direct selection of LTD did not differ substantially between the two nutrient environments. Differential selective effects of growth traits



**Fig. 4** Path diagrams illustrating relations between traits in path analysis model 3 at high (a) and low (b) nutrient supply (see also Table 7). The shaded area represents the complex of early growth traits. Only path coefficients with a ML-estimated value  $\geq 0.20$  are shown. To facilitate comparison of effects, all values are standardized path coefficients, including those leading directly to relative fitness. Note that this is different from the SEM selection measures shown in Table 7, which used unstandardized coefficients obtained from models with relative fitness and standardized phenotypic traits for purposes of comparison with MLR-derived regression coefficients.

	Initial seed weight	RGR	ULR	LAR	SLA	LMF	LTD	Flowering time
Initial seed weight		-0.25	<i>0.13</i>	<b>-0.57***</b>	-0.40*	-0.20	-0.07	<i>0.50**</i>
RGR	-0.34		<b>0.81***</b>	<i>0.20</i>	<i>0.22</i>	<i>0.02</i>	-0.04	<i>0.05</i>
ULR	-0.27	<b>0.73***</b>		-0.39*	-0.11	-0.20	<i>0.14</i>	<i>0.25</i>
LAR	-0.19	<b>0.55***</b>	-0.13		<i>0.48**</i>	<i>0.44*</i>	-0.30	-0.42*
SLA	-0.26	<i>0.40*</i>	-0.16	<b>0.67***</b>		<b>-0.56***</b>	-0.48**	-0.11
LMF	0.08	0.13	0.03	0.34	-0.46**		<i>0.23</i>	-0.29
LTD	-0.51**	-0.11	0.30	-0.48**	-0.37*	-0.08		-0.31
Flowering time	0.20	-0.18	0.20	<b>-0.56***</b>	-0.33	-0.25	0.15	

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Bold values are significant after sequential Bonferroni adjustment within nutrient levels (Rice, 1989).

RGR, relative growth rate; ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; LTD, leaf tissue density.

**Table 6** Correlation matrix, based on accession means, for early growth traits, initial seed weight, and flowering time. Values above the main diagonal (in italic) represent Pearson correlation coefficients under nutrient-poor conditions and values under the diagonal represent correlations under nutrient-rich conditions.

	High nutrients		Low nutrients	
	MLR $\beta$	SEM† Causal effect	MLR $\beta$	SEM Causal effect
<b>Model 1</b>				
Initial seed weight	0.07	0.01	0.07	0.10
Flowering time	-0.06	-0.06	0.16**	0.18
RGR	0.06	0.02	0.06	0.05
<b>Model 2</b>				
Initial seed weight	0.05	0.01	0.07	0.10
Flowering time	0.07	0.02	0.16**	0.20
ULR	-0.07	-0.06	0.07	0.04
LAR	0.21***	0.11	0.05	0.04
<b>Model 3</b>				
Initial seed weight	0.03	0.01	0.06	0.10
Flowering time	0.06	0.01	0.18**	0.19
ULR	-0.06	-0.05	0.07	0.04
SLA	0.20*	0.15	0.02	-0.03
LMF	0.14*	0.05	0.08	-0.00
LTD	-0.05	-0.09	0.01	-0.08

Significance testing for MLR: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

†No significance test for SEM total causal effects of individual traits.

In Multiple Linear Regression (MLR), partial regression coefficients  $\beta$  measure direct linear selection on a trait whereas controlling for variation in all other traits in the model. In path analysis (via Structural Equation Modelling, SEM), direct linear selection is estimated as the sum of all direct and indirect forward paths, or the total causal effect, from the focal trait to fitness according to the path diagram. To avoid problems of collinearity among the variables within the growth complex when fitting their joint effects on flowering and fitness traits, three different models were applied in which RGR was subsequently factorized into its constituent components (with LTD included in the third model as a component trait of SLA; see also Fig. 4).

RGR, relative growth rate; ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; LTD, leaf tissue density.

between nutrient environments could be partly attributed to differential effects of flowering time on fitness. At both high and low nutrient supply, plants that delayed flowering achieved a higher total biomass (data not shown), but only in the low nutrient environment this was associated with high seed production. In the nutrient-rich environment, the positive effect of delayed

flowering via increased total leaf area was counteracted by a negative direct effect on fitness.

## Discussion

In this study, we empirically tested the hypothesis that RGR and its component traits confer differential selective

effects in contrasting nutrient environments. Making use of naturally occurring intraspecific variation in growth traits, and linking this to variation in lifetime fitness measured in controlled nutrient environments, we were able to demonstrate a selective advantage of high SLA and LAR under nutrient-rich conditions. However, we did not detect an effect of variation in RGR on fitness. Low LTD-genotypes were successful under nutrient-rich conditions via correlations with traits under direct selection (e.g. via its effect on SLA) but direct selection on this trait did not differ much between the nutrient environments. In contrast to our expectation, we observed no fitness advantages of low SLA, LMF, and LAR or high LTD values under nutrient-poor conditions. Under these conditions, plant fitness was determined mainly by variation in flowering time and, to a lesser extent, initial seed size.

### Growth trait variation

For most of the growth traits, we detected highly significant inherent differences between populations and/or accessions. The observed among-genotype variance components for growth traits were of comparable magnitude as reported previously for physiological and allocation-related traits of *H. spontaneum* seedlings from various populations in Israel (van Rijn *et al.*, 2000), and allowed for detecting relationships between these traits and fitness based on accession means in the selection analyses. However, among-genotype variance components for RGR and particularly ULR were low and generally not significant, which biased the selection analysis against finding fitness effects of the two traits compared with other traits. These low variance components might be partly explained by inherent inaccuracies in measuring RGR (and ULR, especially problematic for short harvest intervals; see Poorter & Garnier, 1996) as two individual plants are paired to yield one growth score over the harvest interval. For the other traits each individually harvested plant yielded an accurate measure, that was subsequently averaged over two plants of different age to yield one score for the harvest interval. Nevertheless, the accession mean estimates of RGR were sufficiently accurate to detect a significant correlation between RGR scores across the two nutrient treatments ( $r = 0.40$ ,  $n = 32$ ,  $P < 0.05$ ). Additional evidence that the lack of fitness effect of RGR is not because of the absence of among-accession variation in this trait comes from plants that were harvested during a third harvest at day 36. These plants were excluded from the analysis presented in this paper because no accurate leaf area measurements were made, and consequently ULR, LAR and SLA could not be determined. When calculated over the interval between the first and the third harvest, the among-genotype variance component for RGR was highly significant (high-nutrients:  $\sigma_{\text{genotype}}^2 = 56.1\%$ , population effect  $F_{7,92} = 7.2$ ,  $P < 0.001$ ; low-nutrients:  $\sigma_{\text{genotype}}^2 = 34.3\%$ ,

population effect  $F_{7,92} = 5.4$ ,  $P < 0.001$ ). But also using these data no significant effect on fitness was observed in univariate or in multivariate selection analysis (with initial seed size and flowering time in the MLR model). Although selection analyses within environments were somewhat complicated by differing heritable components of variance for the traits, comparing fitness effects of individual traits across environments was not compromised because among-genotype variance components were similar in the two nutrient treatments.

In our study, variation in RGR was governed strongly both by LAR and ULR, of which ULR seemed most closely associated with RGR. Across-species comparisons often indicate LAR as the main determinant of RGR (Poorter & Remkes, 1990; Poorter & van der Werf, 1998). However, the relative importance of LAR and ULR depends on experimental growth conditions (Meziane & Shipley, 1999) and may differ across taxa (Garnier, 1991; but see Poorter & van der Werf, 1998). An equal or even dominant control by ULR when compared with LAR has been reported previously in studies comparing different grass species (Garnier, 1992; Ryser & Wahl, 2001) or intraspecific genotypes (Biere, 1996; Meerts & Garnier, 1996).

### Selection analysis

The observed total fitness effects of some growth traits in this study (as revealed by univariate selection; see Table 5) are in line with across-species correlations of these traits and habitat productivity, which show prevalence of genotypes with high SLA, high LAR, and low LTD in productive habitats (Poorter & Remkes, 1990; Ryser, 1996; Cunningham *et al.*, 1999; Wright & Westoby, 1999; Ryser & Wahl, 2001). This supports the idea that inherent variation in SLA and LAR, as observed among plant species from habitats that differ in productivity, is shaped by natural selection in response to different nutrient availabilities. However, the results suggest that low LTD values may be selected under high nutrient conditions as a correlated response without conferring a direct fitness effect that is either very strong or very different from the low-nutrient environment. As observed in previous studies (Biere, 1996; Cheplick, 2001) differential fitness effects among habitats differing in nutrient richness did not result from low growth trait values being adaptive at limiting nutrient supply, but because high values are beneficial under nutrient-rich conditions. A similar pattern was observed in agricultural varieties of *Brassica campestris*, where high SLA and LAR (leaf investment) conferred a competitive advantage at high nutrient supply, but at low supply the varieties that invested more in root biomass were not favoured (Li *et al.*, 1999). The general failure to demonstrate opposing fitness effects of growth traits might be related to the protective greenhouse environments of these studies. In the absence of herbivory and other disturbances, a

selective advantage of biomass preservation over accumulation of new biomass may not be present under limiting nutrient conditions, whereas this could be more important in nutrient-poor natural environments, as suggested by results from Biere (1996) who showed that low SLA-genotypes of *Lychnis flos cuculi* were favoured under limiting nutrient supply in natural fields but not under greenhouse conditions.

The MLR and path analysis models used to analyse direct selection of growth traits generally agreed quite well, although the estimation of the selective effects differed for some traits (e.g. for LMF; see Table 7). Although path analysis potentially gives more accurate estimates of direct selection because it allows for indirect causal effects via intermediate traits, the validity of the model's selection estimates depends on the accuracy of the defined structure of causality. The accuracy of the model in capturing selection on a trait can be estimated from the discrepancy between the observed and the model-implied correlation of the trait with fitness (Scheiner *et al.*, 2000). This correlation describes total selection, including indirect selection via backward paths from the trait to fitness. In the case of LMF this discrepancy was small (difference between observed and model-implied  $r$ : 0.04 at high nutrients and 0.05 at low nutrients; calculations performed using AMOS 4.01), suggesting that the model captured selection on this trait rather well. But for some other growth traits the discrepancy was considerable (e.g. for LTD and ULR), which indicates that path analysis-estimates of direct selection for these traits can only be interpreted cautiously. Multiple regression models, on the contrary, do not suffer from the problems associated with the path analysis-estimates, but only because they rely on the unrealistic assumption that independent traits are not causally structured, and therefore can exactly recreate the observed correlations.

Contrary to our predictions no direct selection on RGR was observed at high nutrient supply, and differential direct selection on component traits did not result in a correlated response of RGR. In both nutrient environments, total selection of RGR was not significant. While acknowledging that the observed low among-genotype variance components of RGR in our study may have complicated the detection of selective RGR-effects, this lack of a correlated response can be at least partly explained by the negative correlations between the underlying component traits. As observed in this study and in others (Poorter & van der Werf, 1998), negative correlations often exist between SLA and LMF, and between LAR and ULR. These negative correlations buffer RGR against changes in its underlying components (McKenna & Shipley, 1999; Meziane & Shipley, 1999). If selection favours high-SLA genotypes, a fitness effect of RGR-variation may therefore not be observed (above the significance threshold) because these genotypes tend to have low values for LMF and ULR.

It should be noted that our data cannot explain the pattern of observed differences in growth traits among the *H. spontaneum* populations used, and it remains to be investigated if, or to what extent, inherent variation in this model species is actually shaped by adaptation to differing nutrient regimes. Failing to detect a relationship between inherent RGR and habitat fertility among and within *Hordeum* species, Chapin *et al.* (1989) argued that in this genus RGR-variation should not be considered as an adaptation to soil fertility. As information on soil fertility of the populations used in this study is not available, we cannot further explore this hypothesis directly. However, initial seed size and LMF showed associations with moisture and temperature characteristics at the natural growing sites of the populations (January temperature, annual precipitation, humidity and evaporation; see Fig. 1), as indicated by multiple regression models in which population mean trait values for the seven Israeli populations were regressed on these environmental characteristics (initial seed size:  $r^2 = 0.69$ , multiple regression model  $F_{2,4} = 4.6$ ,  $0.05 < P < 0.1$ ; LMF at low-nutrient supply:  $r^2 = 0.81$ , multiple regression model  $F_{2,4} = 8.4$ ,  $P < 0.05$ ; for the analysis, the environmental variables were summarized in two principal components that captured 97% of the variation). This is in agreement with a previously reported relationship between seed size and water availability in *H. spontaneum* (populations from drier environments have smaller seeds; Nevo *et al.*, 1984; Volis *et al.*, 2002a). Such associations could reflect adaptive differentiation, which would indirectly support the view that variation in the RGR-complex as observed among *H. spontaneum* populations should not be solely viewed as an adaptation to differences in soil fertility. Some component traits (e.g. SLA) might be evolutionarily shaped by nutrient-related selection pressures to a considerable extent, but other traits (e.g. LMF) may respond to moisture-related selection pressures as well, either directly or indirectly via correlations with seed size.

The aim of this study was to experimentally test the hypothesis that growth traits confer different selective effects in different nutrient environments. Our results only partly support the view that the often-observed association between habitat richness and growth traits reflects adaptation to different nutrient levels. For some of the traits (SLA, LAR), we demonstrated differences in direct selection among nutrient levels that are in line with observed among-species associations between growth traits and habitat characteristics. But for other traits (LTD) we showed that the observed patterns might be brought about largely by indirect selection. Our results do not confirm that high RGR is targeted by selection under high-nutrient conditions, but they support the idea that not RGR itself but its underlying component traits are targeted by selection differentially in contrasting nutrient environments.

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