

Phenotypic selection and regulation of reproduction in different environments in wild barley

S. VOLIS,^{*}† K. J. F. VERHOEVEN,[‡] S. MENDLINGER^{*} & D. WARD[§]

^{*}The Institutes for Applied Research, Ben-Gurion University of the Negev, Beer Sheva, Israel

[†]Mitrani Department for Desert Ecology, Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boqer, Israel

[‡]Department of Plant Population Biology, Netherlands Institute of Ecology, Nioo-Knaw, Heteren, The Netherlands

[§]Department of Conservation Ecology, Stellenbosch University, Matieland, South Africa

Keywords:

natural selection;
path analysis;
phenotypic integration;
seed abortion;
seed size-number trade-off;
trait correlations

Abstract

Plasticity of the phenotypic architecture of wild barley, *Hordeum spontaneum*, was studied in response to water and nutrient stress. Direct and indirect selection on several vegetative and reproductive traits was estimated and path analysis used to reveal how regulating pathways via maternal investment differed between environments. Vegetative traits displayed differential regulating effect on fitness across experimental environments: (1) increase in size was selected for under optimal conditions and under water stress, but not under nutrient stress; (2) allocation to root biomass was selected for under optimal conditions, but it had no effect under nutrient stress and was strongly selected against when water was limiting; (3) delayed onset of reproduction was selected under nutrient limitation whereas earlier onset was selected under water stress. The regulating effect of reproductive traits on final reproductive output also differed across treatments, operating either at the 'early' stage of plant development through varying the number of initiated spikelets per spike (no stress and water stress treatment) or at the 'late' developmental stage adjusting the fertile spikelet weight (no stress and nutrient stress treatment). Reproductive output was regulated via seed abortion under no stress and water stress treatments. Although the underlying mechanism of the regulation through abortion has yet to be discovered, the specific mechanism of abortion under water stress appears to be different from that under optimal conditions. Our results demonstrate that not only is the character architecture in wild barley plastic and sensitive to changing availability of water and nutrients, but the regulating mechanism of maternal investment is also environmentally sensitive.

Introduction

Phenotypic plasticity has been intensively studied at both univariate (reviewed in Bradshaw, 1965; Schlichting, 1986; Sultan, 1987) and multivariate levels (reviewed in Schlichting & Pigliucci, 1998; see also Pigliucci & Schlichting, 1998; Pigliucci & Marlow, 2001; Pigliucci & Hayden, 2001; Pigliucci & Kolodnynska, 2002) as a means by which an organism can adapt to varying environ-

mental conditions. The multivariate approach views a phenotype as a complex unit in which phenotypic and genetic architectures are environmentally labile. Environment-specific phenotypic responses may involve phenological, vegetative or reproductive characters, from which complicated interrelations (character architecture) can be estimated from correlations among traits and between traits and fitness.

Fitness of annual plants is determined by total reproductive output and the quality of the propagules (Harper *et al.*, 1970; Bazzaz & Reekie, 1985). These two fitness components may have a complicated relationship because of direct and indirect environment-specific

Correspondence: S. Volis, The Institutes for Applied Research, Ben-Gurion University of the Negev, POB 653, Beer Sheva 84105, Israel.
Tel.: 972 8 6477197; fax: 972 8 6472984; e-mail: volis@bgumail.bgu.ac.il

trade-offs during development (McGinley, 1989; Wolfe, 1995; Volis *et al.*, 2002a), and may be determined by other traits not directly related to fitness (e.g. onset of reproduction, plant size, reproductive effort) that also vary across environments (Farris, 1988; Reekie & Bazzaz, 1992; Stöcklin & Favre, 1994; Kudoh *et al.*, 1995; Pigliucci & Schlichting, 1998). For example, early onset of reproduction, small plant size and high reproductive allocation are usually associated with uncertain or stressed environments with a short growing season, while in more predictable and favourable environments, characterized by longer seasons, plants usually reproduce later, after reaching larger size, and allocate less to reproduction (Jones, 1971; Reekie & Bazzaz, 1992).

Understanding the selection process requires: (i) identifying the main evolutionary forces of an environment where a species lives; and (ii) analysing the effects of these forces on individual plant fitness through the changes in plant character architecture. In this paper, we analyse phenotypic selection in wild barley under different environmental conditions. Water and nutrient stresses are among the major environmental factors of either abiotic (drought, leaching of soil) or biotic (depletion of resources because of competition) origin and are likely to induce selective responses. We focused on plasticity in traits that are closely related to fitness and that are involved in local adaptation and formation of ecotypes, viz. onset of reproduction, plant size, root/shoot ratio and maternal investment (Volis *et al.*, 2002a,b,c). Plasticity in maternal investment is of special interest because changes in fruit (seed) number and quality directly influence plant fitness. A widely-held view is that abortion of flowers and developing fruits is the main regulatory pathway of plant reproduction (see e.g. Stephenson, 1981; Burd, 1998). As seed size is one of the least plastic plant characteristics, its importance for regulation of reproduction has been questioned (Harper *et al.*, 1970; Silvertown, 1989).

Existing offspring size optimization models (Smith & Fretwell, 1974; Lloyd, 1987; Geritz, 1995; Rees & Westoby, 1997) are based on the assumption that maternal resources are fixed and that there is a trade-off between the number and size of offspring. However, although some studies provide empirical evidence of a trade-off in resource allocation between seed size and number (Wolf *et al.*, 1986; Lalonde & Roitberg, 1989; Andersson, 1990; Wolfe, 1995), it has not been found in other studies (Marshall *et al.*, 1986; Mazer, 1987; Michaels *et al.*, 1988; Winn, 1988). Nevertheless, as pointed out by Geritz (1995), absence of a negative correlation between seed size and number may be due to variation in a third variable (e.g. plant size) that obscures an existing relationship (Shipley & Dion, 1992; Venable, 1992).

We recently reported a negative correlation between seed mass and number in populations of wild barley distributed along an aridity gradient that appears to

induce two opposite plant strategies: (1) reduction in yield and increase in seed mass with decreasing aridity and (2) increased yield but reduced seed mass with an increase in annual rainfall (Volis *et al.*, 2002a). However, understanding how this trade-off evolves requires a more detailed insight into the causal selection process. This can be done by establishing causal paths relating traits involved in maternal investment regulation (seed mass and number, abortion rate) with fitness, on one hand, and other reproductive, vegetative and phenological traits, on the other hand. A comparison of selection on trait complexes in three environments (optimal, water stress and nutrient stress) is a major objective of this paper. Specific questions addressed are: (1) whether the phenotypic architecture (among trait covariance structure) is affected by the environment and (2) how reproductive output is regulated under different environmental conditions.

Analysis of uni- and multivariate phenotypic plasticity in response to water and nutrient stresses of four ecotypes distributed along an aridity gradient in Israel revealed that plant responses to stress were largely independent of plant origin, with differences between ecotypes under stressful conditions due entirely to differences in amount, but not architecture of plasticity (Volis *et al.*, 2002b). This was the rationale for studying phenotypic selection under optimal and stressed conditions using plants originating from four different environments.

Materials and methods

Study species and choice of populations

Wild barley, *Hordeum spontaneum* Koch, is a predominantly selfing annual grass that occurs in Mediterranean and steppe vegetation types and penetrates into favourable desert microsites, such as ephemeral river valleys (wadis). In the present study, we used plants from four environments in Israel, listed here in order of decreasing aridity: desert (SB), batha (BG), grassland (AM) and mountain (MH) with 90, 400, 600 and 1600 mm mean annual rainfall, respectively. For detailed description of the environments, see Volis *et al.* (2002a).

Experimental design

Four offspring from each of 15 mother plants per population were grown under different water and nutrient regimes. As wild barley is predominantly autogamous (>98%, Nevo *et al.*, 1979) the offspring of each mother plant can be considered genetically nearly identical and represent a single genotype. After emergence four randomly-chosen seedlings per mother plant were transplanted into plastic trays and at the two-leaf stage (in 2 weeks) were transferred singly to 10 L pots containing terra rossa soil with a single plant per pot.

Each of four plants per genotype was subjected to one of the following four regimes:

1. high level of water and nutrients (HH): amount of water equivalent to 500 mm of rainfall during the growing season applied as 1.5 L of water once per week (which is approximately in accord with the natural pattern of precipitation in the Mediterranean region), 10 g of slow-release fertilizer (Osmocote®, containing N 14%, P 14% and K 14% and microelements; The Scotts Company) at the beginning of experiment and 100 mg of 20 : 20 : 20 NPK fertilizer weekly;
2. high level of water and low level of nutrients (HL): the same water treatment as in HH but with no nutrients added (soil was leached prior to experiment and its organic nitrogen content ranged from 0.85 to 1.19 mg kg⁻¹);
3. low level of water and high level of nutrients (LH): the same nutrient treatment as in HH but amount of water equivalent to 150 mm of rainfall during the growing season applied as 100 mL of water once per week plus 1.5 L once per month;
4. low level of water and nutrients (LL): the same water treatment as in LH and the same nutrient treatment as in HL.

The response of plants to the LL was found not to differ from the response to LH and was omitted. With a sample size of 60 plants per treatment we thus analysed a total of 180 plants. The following traits that affect or are involved in maternal investment were analysed: days to anthesis, vegetative biomass, ratio root to vegetative shoot biomass, reproductive effort (ratio biomass of fertile spikelets to total biomass), number of spikelets per spike, number of spikes, fertile spikelet weight, abortion rate (percentage aborted spikelets of the total number of initiated spikelets). The number of spikelets per spike and number of spikes were counted at flowering and together provided the number of initiated spikelets. All spikes had been harvested upon maturation but before shattering and used to obtain per plant abortion rate and average weight of fertile spikelets. Survival under HH, HL and LH treatments till bolting was close to 100%, although not all plants eventually set seed. Nevertheless, they were included in the analyses with fitness = 0.

Data analysis

Total reproduction in plants comprises a number of reproductive components, which in simplest form include: (1) the number of flowers or initiated spikelets; (2) the proportion of flowers or initiated spikelets developed into seeds; (3) the weight of individual seeds. Therefore, a measure of fecundity that estimates fitness must incorporate all the reproductive components. We used the total weight of fertile (shattering) spikelets produced by a plant as an estimate of its fitness (Volis *et al.*, 2002a).

To evaluate total and direct selection on the traits, selection gradients and differentials were calculated for

each treatment separately, using data on individuals of all four ecotypes. Relative fitness was estimated within each treatment as the total weight of mature spikelets produced by a plant relative to the average total weight of mature spikelets per plant in that treatment. Standardized selection differentials were calculated on the traits adjusted to within-treatment standard deviation units as the covariance of relative fitness and the particular trait (CORR procedure in SAS, version 8.2; SAS Institute Inc., Cary, NC, USA; significance of selection differentials was assessed via Pearson correlations). Selection gradients were estimated for two sets of traits (vegetative and reproductive) separately using multivariate regression analysis (Lande & Arnold, 1983). Directional selection gradients (β) were calculated as a partial linear regression coefficient of relative fitness on the standardized traits.

To examine variation in the phenotypic architecture across the treatments we compared the three phenotypic (co)variance matrices using the common principal components method (Flury, 1988). Phenotypic variance-covariance matrices (P-matrices) for the traits adjusted to within-treatment standard deviation were actually the correlation matrices. Three matrices were analysed simultaneously with the null-hypothesis that the matrices are unrelated; the degree of dissimilarity was inferred from the number of principal components that are common. Log-likelihood statistics estimated the fit of the model to the observed matrices and tested the goodness of fit of each model against the model of unrelated structure (jump-up procedure, Phillips & Arnold, 1999). Common principal component analysis was performed using the program CPC (Phillips, 1998).

Path analysis was used to visualize the complex relationship between multiple traits and individual fitness under optimal and stressed environmental conditions (Kingsolver & Schemske, 1991). In this analysis, the path diagram shows an explicit system of linear paths depicting the causal regulating mechanisms. We fitted a model with three hierarchical levels (Fig. 1). In this model, the vegetative traits (aboveground vegetative biomass, root : shoot ratio and days to anthesis) directly influence four reproductive traits (number of spikes, spikelets per spike, fertile spikelet weight and abortion rate), and the latter, in turn, directly affect fitness estimated by total weight of fertile spikelets. All possible correlations between the vegetative traits were modelled. Also, the model allowed for correlated error variables of the reproductive traits, as individual plants may tend to show above-average or below-average values for several of these fitness component traits simultaneously due to factors that are not explicitly specified in the diagram. In all three environments, model χ^2 values were nonsignificant, indicating good agreement of the proposed model with the actually observed covariances among traits (HH: $\chi^2 = 4.6$, $P = 0.20$; HL: $\chi^2 = 1.5$, $P = 0.68$; LH: $\chi^2 = 1.4$, $P = 0.71$). All traits were tested for normality and standardized prior to analysis. The path analysis was

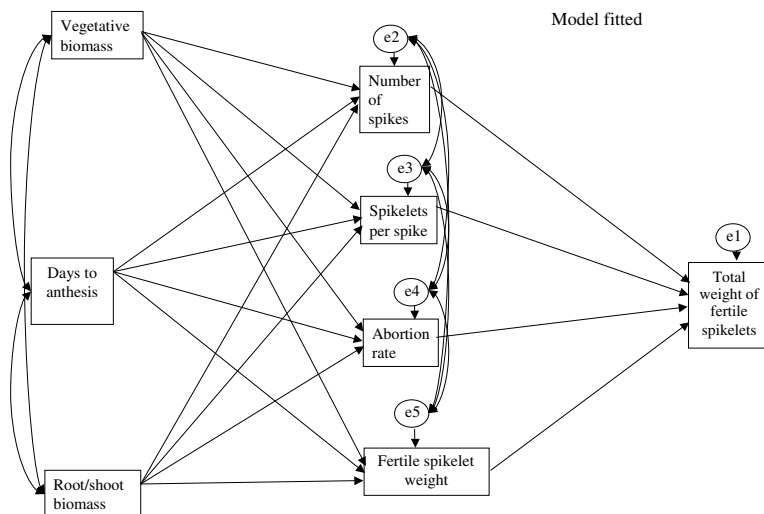


Fig. 1 Path models used for estimating selection in wild barley under three treatments.

performed for each treatment separately using structural equation modelling (CALIS procedure in SAS, version 8.2; SAS Institute Inc.).

Results

Mean phenotypic responses to water and nutrient stress

All measured traits except root/shoot biomass and abortion rate exhibited highly significant differences among treatment means with low water treatment (LH) having a much stronger effect on the traits than low nutrient treatment (HL) (Table 1). Under limited nutrient supply (HL), plants produced fewer spikes and initiated fewer spikelets than plants under optimal conditions (HH), but with no difference in total weight of fertile spikelets between the HL and HH treatments (Table 1). In contrast, limiting water caused a very substantial decrease in number of spikes, spikelets per spike and their product number of initiated spikelets, and total weight of fertile spikelets (Table 1). In addition, vegetative biomass and reproductive effort decreased, and days to anthesis increased under LH treatment (Table 1).

Trait correlations

We estimated the trait phenotypic covariances under the three treatments and a comparison of the HH vs. HL and HH vs. LH correlation matrices revealed a number of correlations that were statistically significant under stress conditions, but not under nonstressed conditions and vice versa (Table 2). There was no correlation between vegetative biomass and root/shoot ratio under nonstressed conditions, while these traits were negatively correlated under both nutrient and water stresses ($r = -0.49$ and $r = -0.62$, $P < 0.001$, respectively).

Abortion rate was positively correlated with days to anthesis ($r = 0.43$, $P < 0.01$), and negatively correlated with spikelets per spike ($r = -0.31$, $P < 0.05$) under water stress, but not under other two treatments ($r = -0.05$ and -0.01 , $r = -0.05$ and 0.04 , n.s., respectively). On the contrary, abortion rate correlated with number of spikes under nonwater stress conditions only, i.e. HH and HL (0.28 and 0.40 , $P < 0.05$ and 0.001 , respectively). The correlation between days to anthesis and number of spikes changed from negative under nonstressed conditions ($r = -0.27$, $P < 0.05$) to positive under nutrient stress ($r = 0.34$, $P < 0.01$).

We tested for differences in the overall pattern of trait correlations between the three environments by comparing the phenotypic correlation matrices. The matrices differed significantly (rejection of equality and also of proportionality with only three principal components shared among matrices; see Table 3).

Selection differentials and gradients

Two reproductive traits, number of spikes and reproductive effort showed highly significant and positive selection differentials under all treatments, but selection differentials of other traits varied across treatments in both strength and sign (Table 4). In the absence of nutrient or water stress, fitness was positively, albeit modestly, associated with increased vegetative biomass, increased allocation to root biomass at the expense of shoot biomass, and increased spikelet number and weight, while abortion was selected against (Table 4). Under nutrient stress, selection differentials for vegetative biomass, root/shoot biomass ratio, number of spikelets per spike and abortion rate were not significant. However, a strong positive selection differential was observed for days to anthesis (Table 4). Under water stress, increased vegetative biomass was selected for, and

Table 1 *F*-statistics for one-way ANOVA and mean (\pm SE, *n* below) trait values in different environmental treatments

Traits	F (d.f. 2, 167–175)	Treatment		
		HH	HL	LH
Vegetative biomass (g)	73.0***	13.37 \pm 0.53 ^a 55	12.17 \pm 1.57 ^a 60	5.17 \pm 0.67 ^b 60
Root/shoot biomass	1.3 ns	0.17 \pm 0.01 54	0.25 \pm 0.02 60	0.32 \pm 0.07 60
Days to anthesis	11.9***	116.6 \pm 1.2 ^a 59	114.0 \pm 1.1 ^a 60	122.8 \pm 1.6 ^b 53
Reproductive effort	38.2***	0.26 \pm 0.01 ^a 55	0.26 \pm 0.01 ^a 60	0.12 \pm 0.02 ^b 60
Spikelets per spike	76.7***	18.56 \pm 0.49 ^a 59	19.63 \pm 0.45 ^a 60	11.84 \pm 0.48 ^b 53
Number of spikes	91.5***	10.09 \pm 0.51 ^a 57	8.30 \pm 0.46 ^b 60	2.30 \pm 0.18 ^c 53
Number of spikelets initiated	103.3***	135.1 \pm 8.16 ^a 58	109.9 \pm 5.79 ^b 60	21.2 \pm 2.45 ^c 60
Spikelet weight (g)	7.1***	0.057 \pm 0.002 ^{ab} 58	0.065 \pm 0.003 ^a 60	0.051 \pm 0.002 ^b 52
Abortion rate	2.8**	0.25 \pm 0.02 58	0.18 \pm 0.02 60	0.26 \pm 0.03 53
Total weight of fertile spikelets (g)	129.6***	5.44 \pm 0.33 ^a 58	5.25 \pm 0.22 ^a 60	0.75 \pm 0.10 ^b 60

*** $P < 0.001$, ** $P < 0.10$, ns, not significant.

HH, high water high nutrients; HL, high water low nutrients; LH, low water low nutrients. Shared letters within each row indicate that means are not significantly different as revealed by Tukey's test at $P < 0.05$.

allocation to aboveground vegetative biomass at the expense of root biomass was favoured (Table 4). Selection for reproductive traits under water stress showed a similar pattern as under no stress, with the exception of spikelet weight that had no significant association with fitness. Strong selection towards increase in spikelet number per spike was observed. Delay in reproduction, as indicated by days to anthesis, was selected against (Table 4).

In the multivariate analyses, the three vegetative traits (vegetative biomass, R/S ratio and days to anthesis) together explained 38, 20 and 27% of the variance in total weight of fertile spikelets under HH, HL and LH treatments ($P < 0.001$, 0.01 and 0.01, respectively).

The multiple regression of relative fitness (total weight of fertile spikelets) on vegetative traits indicated directional selection for increased vegetative biomass in the unstressed and water-stressed environments, but not under nutrient stress (Table 4). Positive directional selection gradient was detected for root biomass allocation only in the unstressed environment. The fitness effect of time to anthesis differed between treatments: delayed flowering conferred a fitness advantage under nutrient stress while it conferred a disadvantage under water stress (Table 4).

The multiple regression of relative fitness on reproductive traits (which themselves are components of fitness) showed general congruence of their effects on the final estimate of fitness with relatively small differ-

ences in the magnitude of the effects. All reproductive traits had highly significant positive effects on total weight of fertile spikelets (except abortion rate) under all three treatments (Table 4).

As trait correlations with final fitness (selection differentials) for some traits were different from the corresponding partial regression coefficients for those traits (selection gradients), the effect of these traits on fitness was partly indirect and mediated by other traits.

Path-analysis of selection

Visual inspection of path coefficients revealed how vegetative traits affected fitness via intermediate reproductive traits, and via correlations with other vegetative traits (which indicate causes of indirect selection). Path coefficients of the reproductive traits to fitness were similar in all environments, with strong positive effects of spike number and spikelet weight, and a strong negative effect of abortion rate (Fig. 2: HH, HL and LH). Note that these path coefficients from fitness components to final fitness are comparable in their pattern with the selection gradients of these traits (cf. Table 4), but different in their absolute values because of the use of standardized final fitness in path analysis and unstandardized final fitness in selection gradient estimation.

Some important paths from vegetative traits to intermediate reproductive traits appeared unaffected by the

Table 2 Phenotypic correlations among different traits in the three experimental treatments (see Table 1 for further explanations)

Treatment (size)	Vegetative biomass	Days to anthesis	Root/shoot weight	Reproductive effort	Number of spikes	Spikelets per spike	Abortion rate	Spikelet weight
HH (<i>n</i> = 57)								
Vegetative biomass	1							
Days to anthesis	-0.04	1						
Root/shoot weight	-0.07	0.41**	1					
Reproductive effort	-0.46***	-0.10	0.19	1				
Number of spikes	0.66***	-0.27*	0.20	-0.04	1			
Spikelets per spike	0.06	-0.65***	-0.02	0.23	0.31*	1		
Abortion rate	0.25	-0.05	0.13	-0.56***	0.28*	-0.05	1	
Spikelet weight	-0.09	0.46***	0.37**	0.35**	-0.15	-0.35**	-0.07	1
HL (<i>n</i> = 60)								
Vegetative biomass	1							
Days to anthesis	0.17	1						
Root/shoot weight	-0.49***	0.36**	1					
Reproductive effort	-0.65***	0.02	0.29*	1				
Number of spikes	0.40**	0.34**	0.01	-0.04	1			
Spikelets per spike	0.03	-0.53***	-0.11	-0.07	-0.11	1		
Abortion rate	0.13	-0.01	-0.04	-0.26*	0.40**	0.04	1	
Spikelet weight	-0.23	0.31*	0.24	0.41**	-0.39**	-0.42**	-0.17	1
LH (<i>n</i> = 56)								
Vegetative biomass	1							
Days to anthesis	-0.05	1						
Root/shoot weight	-0.62***	0.27*	1					
Reproductive effort	-0.22	-0.15	0.04	1				
Number of spikes	0.66***	-0.19	-0.36**	-0.12	1			
Spikelets per spike	0.10	-0.49***	-0.03	0.25	0.26	1		
Abortion rate	-0.01	0.43**	0.15	-0.32*	0.07	-0.31*	1	
Spikelet weight	-0.15	0.45***	0.28*	0.50***	-0.17	-0.18	0.16	1

****P* < 0.001, ***P* < 0.01, **P* < 0.05; the values significant after sequential Bonferroni correction (Rice, 1989) are in bold.

HH, high water high nutrients; HL, high water low nutrients; LH, low water low nutrients.

Table 3 Comparison of correlation matrices among three experimental treatments. χ^2 values were estimated from parametric procedure based on individual values using CPC (Phillips, 1998)

Hypothesis	d.f.	χ^2	<i>P</i> -value
Equality	72	137.6	<0.0001
Proportionality	70	135.7	<0.0001
Full CPC	56	87.7	0.0043
CPC (6)	54	87.1	0.0029
CPC (5)	50	82.8	0.0024
CPC (4)	44	74.1	0.0030
CPC (3)	36	45.2	0.1399
CPC (2)	26	39.0	0.0491
CPC (1)	14	16.3	0.2943
Unrelated			

water and nutrient treatments. In all environments, we observed: (a) a strong positive effect of vegetative biomass on spike number; (b) a negative effect of delayed flowering on the number of spikelets per spike and a positive effect of delayed flowering on spikelet weight; (c) a positive effect of root allocation on the number of spikelets per spike and (d) a positive correlation between

delayed flowering and root allocation (Fig. 2: HH, HL and LH).

Environment-specific path coefficients in the non-stressed environment included a positive effect of vegetative biomass on abortion, a negative effect of delayed flowering on spike number, and positive effects of root allocation on spike number, spikelet weight and abortion rate (Fig. 2: HH). In contrast to the other treatments, a positive effect of vegetative biomass on the number of spikelets per spike was absent in the nonstressed environment, as was a strong negative correlation between vegetative biomass and root allocation (Fig. 2).

Under nutrient stress, but not in the other environments, vegetative biomass had a strong negative effect on spikelet weight (Fig. 2: HL). Delayed flowering had a positive effect on spike number under nutrient stress. Correlated error variables of the reproductive traits indicated that a negative association between spikelet weight and spike or spikelet number, and a negative association between spike number and abortion rate, were partly caused by factors that were not specified in the model. This appeared to be the case in the nutrient-stress treatment only (Fig. 2).

Table 4 Standardized selection differentials and gradients (\pm SE) in the three experimental treatments (cf. Table 1) ($n = 52-60$)

Traits	Standardized selection differentials			Standardized linear selection gradients		
	HH	HL	LH	HH	HL	LH
Vegetative						
Vegetative biomass	0.14*	0.04	0.50***	0.14 \pm 0.06*	0.04 \pm 0.05	0.48 \pm 0.15**
Root/shoot weight	0.13*	0.06	-0.36**	0.18 \pm 0.06**	0.03 \pm 0.05	0.16 \pm 0.18
Days to anthesis	-0.06	0.14***	-0.31*	-0.09 \pm 0.06	0.12 \pm 0.05**	-0.32 \pm 0.13*
Reproductive						
Number of spikes	0.23***	0.15***	0.63***	0.30 \pm 0.04***	0.31 \pm 0.03***	0.68 \pm 0.08***
Spikelets per spike	0.15*	-0.04	0.46***	0.11 \pm 0.04**	0.11 \pm 0.03***	0.28 \pm 0.08**
Spikelet weight	0.14*	0.12**	0.17	0.21 \pm 0.03***	0.26 \pm 0.03***	0.39 \pm 0.08***
Abortion rate	-0.17**	-0.03	-0.32*	-0.24 \pm 0.03***	-0.11 \pm 0.03***	-0.42 \pm 0.09***
Allocation						
Reproductive effort	0.29***	0.19***	0.48***			

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; the values significant after sequential Bonferroni correction (Rice, 1989) are in bold.

HH, high water high nutrients; HL, high water low nutrients; LH, low water low nutrients.

Under water stress, but not in the other environments, a positive effect of delayed flowering on abortion rate was observed. In this environment, delayed flowering showed no effect on spike number (Fig. 2: LH).

Discussion

A combination of several analyses (correlations, univariate and multivariate regression, and path analysis) is a useful approach for getting a detailed view of selection processes in the same data set. However, despite a general agreement, interpretation of the paths in the path diagrams did not always agree with the conclusions derived from trait covariances, selection differentials and gradients. Therefore the interpretation of our results is based, first of all, on the latter, with the path diagrams being rather a pictorial guide to interrelations among traits.

The results show that, under all experimental treatments, an increase in overall plant size, as indicated by vegetative biomass, leads to an increase in the number of reproductive tillers and total number of initiated spikelets (number of spikes multiplied by spikelets per spike). This is a common pattern of relationship between the number of flowers developed and plant size, which sets an upper limit to potential fecundity (Harper & Wallace, 1987). However, the pathway of regulation of reproductive output through investment in vegetative biomass was not the same across the treatments.

When nutrient and water supply is optimal, total selection favours an increase in plant size, which directly increases the number of reproductive tillers, but also increases abortion rate. Under these conditions, despite a high cost of abortion that diminishes the return per unit investment, the relatively low magnitude of the effect of plant size on abortion rate makes an increase in size still

profitable and selected for (Table 4 and Fig. 2: HH). Regulation of maternal investment is achieved through adjustment of such reproductive traits as number of spikelets in a spike (at an early stage), weight of spikelets (at later stage of maturation), and abortion rate, which is negatively related to reproductive effort. High reproductive effort is selected for, but, because environmental conditions provide no cues for resource depletion, inappropriate decisions, with over-commitments in resource allocation, may occur. When too many spikes or spikelets are initiated than a plant can support even under optimal conditions, abortion rate is a means to regulate yield.

When water, but not nutrients, is limiting, total selection maximizes two components of maternal investment: number of reproductive tillers and spikelets per spike, but not the weight of fertile spikelets. Abortion under limited water supply has a high cost, but because of lack of covariance with vegetative biomass (Table 2), increase in the latter is favoured. Although the path diagram suggests that the regulation of maternal investment under water stress is similar to that under no stress, the traits' correlations and the lack of significant total selection on spikelet weight may indicate that the specific mechanism of abortion under water stress is different from the mechanism that is operative under nonstress conditions. In the absence of water or nutrient stress, only some spikelets within a spike (those that are filled later than others) are aborted because of either developmental constraints or competition for resources, while under water stress, when triggering to senescence occurs, total spikes are aborted (those that are initiated later than others – S. Volis, personal observations). In the latter case the number of aborted spikes will depend on the time when senescence starts and not on the number of spikes initiated on a plant. This suggestion is supported by the lack of a significant correlation between spikelet weight

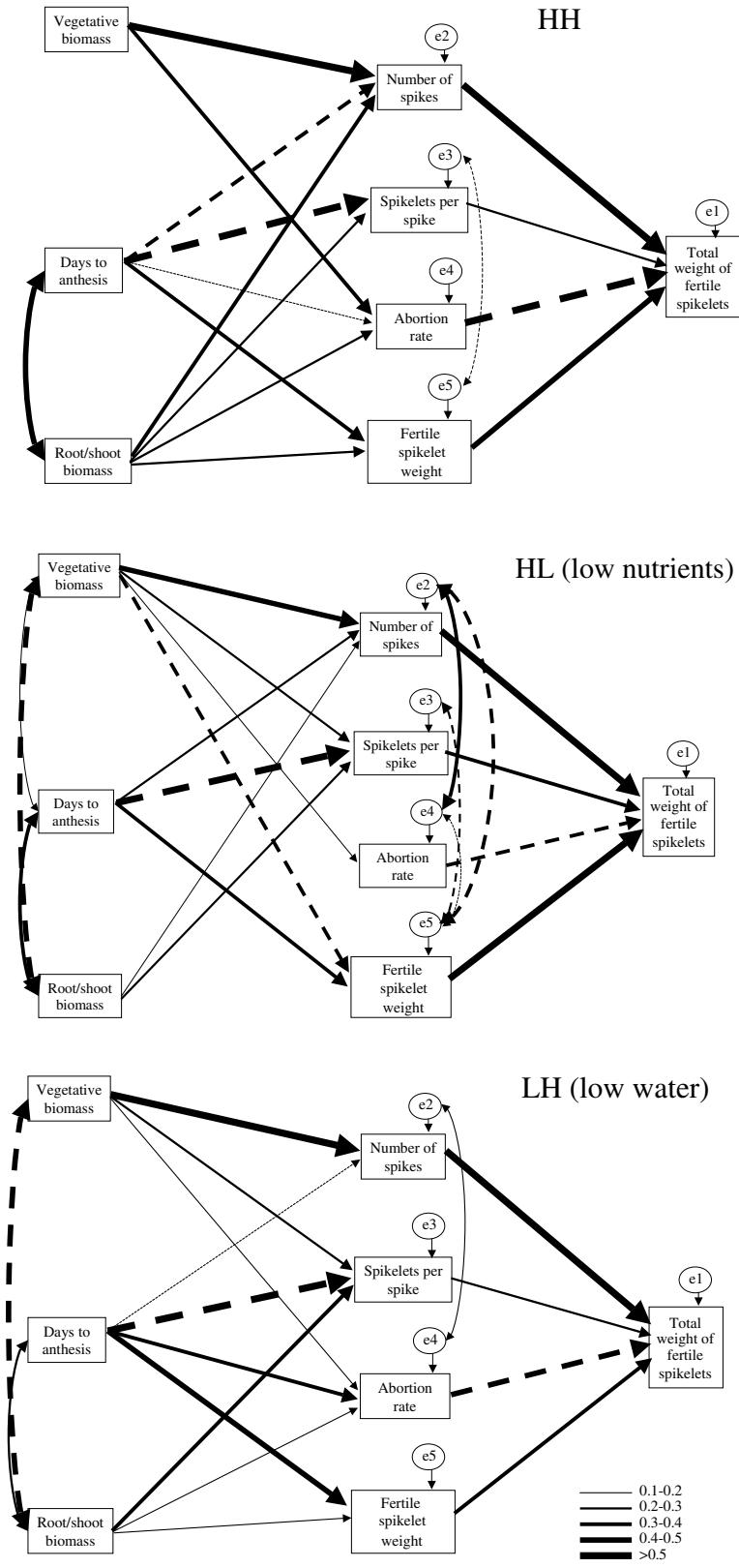


Fig. 2 Path analysis of selection under three treatments: optimal (HH), nutrient stress (HL) and water stress (LH). Solid and dashed lines denote positive and negative relationship, respectively.

and number of spikelets in a spike, on one hand, and between number of spikes and abortion rate, on the other hand. Both these correlations were significant under nonstress conditions.

Some personal observations of the life history of wild barley also support our hypothesis of several abortion mechanisms. In Mediterranean annuals, amount and timing of rainfall events are the main regulating factors of their development, and onset of dry season is a trigger for plant senescence. However, when the rainy season is prolonged and timing of water availability is optimal – a situation mimicked by the HH treatment – resource investment during grain filling may be gradual, with the largest spikelets being filled first. Such intra-plant variation may have both a temporal and plant positional origin. Seeds that are formed earlier may be larger than those formed later because indeterminate growth may diminish the amount of resources available to reproductive organs through time, changing seed weight from the top of inflorescence downwards (Cavers & Steel, 1984; Matthies, 1990; Wolfe, 1995; Torres & Galetto, 1999). Abortion at a relatively late stage of development as a means of regulation of maternal investment appears to evolve if optimization at early stages fails. This may happen under both optimal and water stress conditions, but apparently is less common under the latter as number of initiated spikes under water stress is already very small (two on average vs. 10 under no stress). Indeed, the lowest percentage of aborted spikelets under the LH treatment was detected in the desert ecotype, which usually experiences water stress during its life span (Volis *et al.*, 2002b).

When nutrients, but not water, are limiting, the net selective effect of plant size variation was negligible. Although a direct negative effect of abortion rate on fitness under nutrient stress is only slightly lower than under optimal conditions, the total selection on abortion rate disappears under limited nutrient supply. Regulation of maternal investment under HL can be achieved through spikelet weight, because of intra-plant competition for resources similar to that described for HH. An important difference is, however, that an increase in the number of spikelets per spike is not profitable when nutrients are limited. Under these conditions, any increase in number of spikelets will be compensated by a decrease in their weight, with no change in the total weight of fertile spikelets.

Direct selection on root/shoot ratio was observed only under conditions of no stress and the total selection on root/shoot ratio was positive under HH, not significant under HL and negative under LH, displaying plastic allocation in aboveground vs. root biomass across treatments. Disproportional allocation into root biomass is no longer profitable when nutrients are limiting and is strongly selected against when water is limiting. This appears to indicate that when root/shoot allocation is already high (as is under nutrient stress and especially

under water stress) allocation to more roots does not increase plant fitness.

Transition to reproduction also displayed a plastic correlative response across treatments. Delayed onset of reproduction caused an invariant decrease in number of spikelets per spike and an increase in spikelet weight in all treatments. But only under limited water supply did onset of reproduction have a direct and total negative selection effect on fitness, favouring early onset of reproduction. Earlier we reported (Volis *et al.*, 2002b) that water stress significantly delayed onset of reproduction, a pattern found for other annuals from arid environments (Mott & McComb, 1975; Fox, 1990; van Rooyen *et al.*, 1991; Steyn *et al.*, 1996). The results of our selection analysis suggest that, although water stress causes a delay of reproduction in comparison with an optimal environment because of physiological constraints that limited water supply imposes on plant development, plants that initiate reproduction earlier are favoured. An advantage of early flowering under low and unpredictable water was experimentally demonstrated in a previously reported reciprocal transplant experiment, where wild barley plants of desert origin had higher fecundity at the desert site than those originating in less xeric Mediterranean environment (Volis *et al.*, 2002c). This conclusion is congruent with that of Stanton *et al.* (2000), i.e. that phenotypic selection under water stress favours stress-avoidance traits such as earlier flowering, rather than traits characteristic of stress-tolerance, such as slow growth and delayed reproduction. In contrast, direct and total selection for delayed reproduction was found under conditions of nutrient limitation, a pattern found also by Verhoeven *et al.* (2004).

Regulation of fecundity at different levels of reproductive development has been reported earlier by several authors (Marshall *et al.*, 1986; Harper & Wallace, 1987; Agren, 1989; Matthies, 1990; Stöcklin & Favre, 1994). Our results suggest that, in wild barley, reproductive investment is differently regulated when either nutrients or water are limiting: by varying the size of the seeds (under nutrient but not water stress) or optimizing the number of spikelets in a spike (under water but not nutrient stress). Moreover, although we are still far from complete understanding of how variation in spikelet weight within and among spikelets evolves, selection on spikelet weight appears to be environmentally dependent and presumably regulated through different mechanisms.

Our results demonstrate that not only the character architecture in wild barley is plastic and sensitive to changing availability of water and nutrients, but that the regulating mechanism of maternal investment is also environmentally sensitive. We found, like other studies (Hebert *et al.*, 1994; Schlichting & Pigliucci, 1995; Pigliucci & Schlichting, 1998), that structure of phenotypic correlation matrices is sensitive to environmental conditions where the plants are grown. It is not single traits but

rather a particular combination of character correlations that appear to evolve under environmentally-specific selection, thereby providing the basis for local adaptation.

Acknowledgments

We thank Kamshat Keldibekov, Maxim Madorsky, Edward Berezovsky for assistance in the greenhouse and Ruti Soto for laboratory work. We are also grateful to Peter van Tienderen, and two anonymous referees whose comments helped to improve this paper. A grant from the Israel Academy of Sciences (86293101) supported this study.

This is publication number 423 of the Mitrani Department for Desert Ecology.

References

- Agren, J. 1989. Seed size and number in *Rubus chamaemorus*: between-habitat variation, and effects of defoliation and supplement pollination. *J. Ecol.* **77**: 1080–1092.
- Andersson, S. 1990. Paternal effects on seed size in a population of *Crepis tectorum* (Asteraceae). *Oikos* **59**: 3–8.
- Bazzaz, F.A. & Reekie, E.G. 1985. The meaning and measurement of reproductive effort in plants. In: *Studies on plant demography: a festschrift for John L. Harper* (J. White, ed.), pp. 203–227. Academic Press, London.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genetics* **13**: 115–155.
- Burd, M. 1998. "Excess" flower production and selective fruit abortion: a model of potential benefits. *Ecology* **79**: 2123–2132.
- Cavers, P.B. & Steel, M.G. 1984. Patterns of change in seed weights over time on individual plants. *Am. Naturalist* **124**: 324–335.
- Farris, M.A. 1988. Quantitative genetic variation and natural selection in *Cleome serrulata* growing along a mild soil moisture gradient. *Can. J. Botany* **66**: 1870–1876.
- Flury, B. 1988. *Common Principal Components and Related Multivariate Models*. Wiley, New York.
- Fox, G.A. 1990. Components of flowering time variation in a desert annual. *Evolution* **44**: 1404–1423.
- Geritz, S.A.H. 1995. Evolutionary stable seed polymorphism and small-scale spatial variation in seedling density. *Am. Naturalist* **146**: 685–707.
- Harper, J.L., Lovell, P.H. & Moore, K.G. 1970. The shape and sizes of seeds. *Ann. Rev. Ecol. Systematics* **1**: 327–356.
- Harper, J.L. & Wallace, H.L. 1987. Abortion and the costs of fecundity in *Epilobium montanum* L. *Oecologia* **74**: 31–38.
- Hebert, D., Faure, S. & Olivieri, I. 1994. Genetic, phenotypic, and environmental correlations in black medic, *Medicago lupulina* L., grown in three different environments. *Theor. Appl. Genet.* **88**: 604–613.
- Jones, M.E. 1971. The population genetics of *Arabidopsis thaliana*. II. Population structure. *Heredity* **27**: 51–58.
- Kingsolver, J.G., & Schemske, D.W. 1991. Path analyses of selection. *Trends Ecol. Evol.* **6**: 276–280.
- Kudoh, H., Ishiguri, Y. & Kawano, S. 1995. Phenotypic plasticity in *Cardamine flexuosa*: variation among populations in plastic response to chilling treatments and photoperiods. *Oecologia* **103**: 148–156.
- Lalonde, R.G. & Roitberg, B.D. 1989. Resource limitation and offspring size and number trade-offs in *Cirsium arvense* (Asteraceae). *Am. J. Botany* **76**: 1107–1113.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Lloyd, D.G. 1987. Selection of offspring size at independence and other size-versus-number strategies. *Am. Naturalist* **129**: 800–817.
- Marshall, D.L., Levin, D.A. & Fowler, N.L. 1986. Plasticity of yield components in response to stress in *Sesbania macrocarpa* and *Sesbania vesicaria* (Leguminosae). *Am. Naturalist* **127**: 508–521.
- Matthies, D. 1990. Plasticity of reproductive components at different stages of development in the annual plant *Thlaspi arvense* L. *Oecologia* **83**: 105–116.
- Mazer, S.J. 1987. The quantitative genetics of life history and fitness components of in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed mass variation. *Am. Naturalist* **130**: 891–914.
- McGinley, M.A. 1989. Within- and among plant variation in seed mass and pappus size in *Tragopogon dubius*. *Can. J. Botany* **67**: 1298–1304.
- Michaels, H.G., Benner, B., Hartgerink, A.P., Lee, T.D., Rice, S., Wilson, M.F. & Bertin, R.I. 1988. Seed size variation: magnitude, distribution, and ecological correlates. *Evol. Ecol.* **2**: 157–166.
- Mott, J.J. & McComb, A.J. 1975. Effects of moisture stress on the growth and reproduction of three annual species from an arid region of Western Australia. *J. Ecol.* **63**: 825–834.
- Nevo, E., Zohary, D., Brown, A.H.D. & Haber, M. 1979. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. *Evolution* **33**: 815–833.
- Phillips, P.C. 1998. *CPC: Common Principal Component Analysis*. University of Texas, Arlington. Software available at <http://www.uta.edu/biology/phillips/software>.
- Phillips, P.C. & Arnold, S.J. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* **53**: 1506–1515.
- Pigliucci, M. & Hayden, K. 2001. Phenotypic plasticity is the major determinant of changes in phenotypic integration in *Arabidopsis*. *New Phytologist* **152**: 419–430.
- Pigliucci, M. & Kolodnynska, A. 2002. Phenotypic plasticity to light intensity in *Arabidopsis thaliana*: invariance of reaction norms and phenotypic integration. *Evol. Ecol.* **16**: 27–47.
- Pigliucci, M. & Marlow, E.T. 2001. Differentiation for flowering time and phenotypic integration in *Arabidopsis thaliana* in response to season length and vernalization. *Oecologia* **127**: 501–508.
- Pigliucci, M. & Schlichting, C.D. 1998. Reaction norms of *Arabidopsis*. V. Flowering time controls phenotypic architecture in response to nutrient stress. *J. Evol. Biol.* **11**: 285–301.
- Reekie, E.G. & Bazzaz, F.A. 1992. Cost of reproduction as reduced growth in genotypes of two congeneric species with contrasting life histories. *Oecologia* **90**: 21–26.
- Rees, M. & Westoby, M. 1997. Evolution of seed size in a simple ecological model. *Oikos* **78**: 116–126.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Ann. Rev. Ecol. Systematics* **17**: 667–693.

- Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates, Sunderland, MA, USA.
- Schlichting, C.D. & Pigliucci, M. 1995. Lost in phenotypic space: environment dependent morphology in *Phlox drummondii* (Polemoniaceae). *Int. J. Plant Sci.* **156**: 542–546.
- Shiple, B. & Dion, J. 1992. The allometry of seed production in herbaceous angiosperms. *Am. Naturalist* **139**: 467–483.
- Silvertown, J.W. 1989. The paradox of seed size and adaptation. *Trends Ecol. Evol.* **4**: 24–26.
- Smith, C.C. & Fretwell, S.D. 1974. The optimal balance between size and number of offspring. *Am. Naturalist* **108**: 499–506.
- Steyn, H.M., Van Rooyen, N., Van Rooyen, M.W. & Theron, G.K. 1996. The phenology of Namaqualand ephemeral species. The effect of water stress. *J. Arid Environ.* **33**: 49–62.
- Stanton, M.L., Roy, B.A. & Thiede, D.A. 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution* **54**: 93–111.
- Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Ann. Rev. Ecol. Systematics* **12**: 253–279.
- Stöcklin, J. & Favre, P. 1994. Effects of plant size and morphological constraints on variation in reproductive components in two related species of *Epilobium*. *J. Ecol.* **82**: 735–746.
- Sultan, S.E. 1987. Evolutionary implications of phenotypic plasticity in plants. *Evol. Biol.* **21**: 127–178.
- Torres, C. & Galetto, L. 1999. Factors constraining fruit set in *Mandevilla pentlandiana* (Apocynaceae). *Bot. J. Linn. Soc.* **129**: 187–205.
- van Rooyen, M.W., Grobbelaar, N., Theron, G.K. & van Rooyen, N. 1991. The ephemerals of Namaqualand: effects of photoperiod, temperature and moisture stress on development and flowering of three species. *J. Arid Environ.* **19**: 133–145.
- Venable, D.L. 1992. Size-number trade-offs and the variation of seed size with plant resource status. *Am. Naturalist* **140**: 287–304.
- Verhoeven, K.J.F., Biere, A., Nevo, E. & van Damme, J.M.M. 2004. Differential selection of growth rate-related traits in wild barley, *Hordeum spontaneum*, in contrasting greenhouse nutrient environments. *J. Evol. Biol.* **17**: 184–196.
- Volis, S., Mendlinger, S. & Ward, D. 2002a. Differentiation in populations of *Hordeum spontaneum* Koch along a gradient of environmental productivity and predictability: life history and local adaptation. *Biol. J. Linn. Soc.* **77**: 479–490.
- Volis, S., Mendlinger, S. & Ward, D. 2002b. Differentiation in populations of *Hordeum spontaneum* Koch along a gradient of environmental productivity and predictability: plasticity in response to water and nutrient stress. *Biol. J. Linn. Soc.* **75**: 301–312.
- Volis, S., Mendlinger, S. & Ward, D. 2002c. Adaptive traits of wild barley plants of Mediterranean and desert origin. *Oecologia* **133**: 131–138.
- Winn, A.A. 1988. Ecological and evolutionary consequences of seed size in *Prunella vulgaris*. *Ecology* **68**: 1537–1544.
- Wolf, L.L., Hainsworth, F.R., Mercier, T. & Benjamin, R. 1986. Seed size variation and pollinator uncertainty in *Ipomopsis aggregata* (Polemoniaceae). *J. Ecol.* **74**: 361–371.
- Wolfe, L.M. 1995. The genetics and ecology of seed size variation in a biennial plant, *Hydrophyllum appendiculatum* (Hydrophyllaceae). *Oecologia* **101**: 343–352.

Received 5 June 2003; revised 1 March 2004; accepted 2 March 2004