

# Differentiation along a gradient of environmental productivity and predictability in populations of *Hordeum spontaneum* Koch: multilevel selection analysis

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A contextual analysis combined with path analysis was applied to detect ecotype-specific past selection in hierarchically structured populations of wild barley, *Hordeum spontaneum*. In our analysis a multiple regression model incorporated several individual and ecotype-level unmeasured (derived) traits obtained by factor analysis from 20 measured morphological and phenological traits. Under favourable conditions (high water and nutrients) both individual and ecotype plant size (RF1) were significant predictors of individual plant fitness, estimated by either reproductive biomass or yield. Both individual and ecotype size of reproductive structures (RF2) were significantly related to individual reproductive biomass. Individual yield, however, significantly correlated with ecotype RF2 only. Transition to reproduction (RF3) correlated with neither reproductive biomass nor yield at individual level, but correlated with two estimates of fitness at ecotype level. In all cases, selection at the individual and ecotype levels was in opposition. We interpret the observed effect of ecotype identity on individual fitness not as a current group selection, but as a constraining effect of ecotype-specific past selection. The four ecotypes went through an environmentally specific selection process in their own environments with the optimal strategy evolved. Consequently, this strategy may have a constraining effect on plant performance in other environments. Under conditions of either low water or low nutrients the ecotype level did not contribute to individual fitness. The latter may suggest that a mechanism for plant responses to stress is largely independent of plant origin, with a difference between ecotypes under stressful conditions due entirely to the difference in amount, not architecture, of plasticity. © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2002, 75, 313–318.

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## INTRODUCTION

Understanding the causes of genetic and phenotypic variation, and the mechanisms of its maintenance in natural populations, requires measurements of selection on traits that affect individual fitness. A selection-gradient analysis introduced by Lande &

Arnold (1983) has become a widely used method for assessment of phenotypic selection (Kalisz, 1986; Stratton, 1992; Grant & Grant, 1995; Gross *et al.*, 1998). Gradient analysis, however, assumes that a population is homogeneous with respect to the selection pattern acting on individual characters. If a species is hierarchically substructured into many populations distributed in a heterogeneous environment, population selection processes may differ across a species range. Heisler & Damuth (1987) proposed

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a method that allows one to partition multilevel natural selection into individual and contextual components (i.e. selection due to properties of individual, and selection due to properties of population). In this method, 'contextual' group traits (e.g. size) which may influence fitness are included in a model of multiple regression analysis examining the effects of a suit of traits on individual fitness. A discontent between individual and population regression coefficients is an indication of interpopulation differences in patterns of selection.

Interpopulation differences along broad environmental gradients were detected in several studies (Grant & Wilken, 1988; Ehleringer & Clark, 1988; Macdonald & Chinnappa, 1988, 1989). We are, however, unaware of any study where a formal selection analysis was conducted to reveal the causes of such variation. Nevertheless, clarification of selection processes that lead to formation of ecotypes within a species may allow one to identify major selection pathways resulting in sets of 'coadapted traits designed by natural selection, to solve particular ecological problems' (tactics *sensu* Stearns, 1976).

## MATERIAL AND METHODS

### STUDIED SPECIES AND CHOICE OF POPULATIONS

Wild barley, *Hordeum spontaneum* Koch, is a winter annual and a predominately selfing grass (Harlan & Zohary, 1966). In Israel, despite its mainly Mediterranean and Irano-Turanian distribution in steppe-like formations, wild barley penetrates into desert (<200 mm annual rainfall) and mountain (up to 1600 m elevation) environments where it maintains stable populations. The four populations of *H. spontaneum* used in this study represent four environments in order of increasing rainfall and its predictability: desert (SB), batha (BG), grassland (AM) and mountain (MH), with 90, 400, 600 and 1600 mm of rainfall per annum, respectively. In addition, in desert and mountain localities, plants are exposed to contrasting stresses (drought in the desert, frost in the mountain region). For detailed descriptions of four ecotype environments see Volis (2001).

### EXPERIMENTAL DESIGN

Four offspring from each of 15 mother plants per population (referred here as 'ecotype') were grown under different water and nutrient regimes. As wild barley is predominantly autogamous (98% or more, Nevo *et al.*, 1979), the offspring of each mother plant can be considered genetically identical and represent a single genotype. The four regimes were: (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 litre of water once per week (which is more or less in accord with the natural pattern of precipitation in Mediterranean region), 10 g of slow fertilizer at the beginning of experiment and 100 mg of 20:20:20 (NPK) weekly; (2) high level of water and low level of nutrients (HL), the same water treatment as in HH but with no nutrients added; (3) low level of water and high level of nutrients (LH). The same nutrient treatment as in HH but the amount of water is equivalent to 150 mm of rainfall during the growing season applied as 100 mL of water once per week plus 1.5 L once a 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 following traits were measured: days to awn appearance, days to anthesis, tiller height, number of nodes, flag leaf length and width, penultimate leaf length and width, awn length, spike length, number of spikelets per spike, number of spikes, yield (total number of spikelets produced), reproductive biomass, spikelet weight, root biomass, ratio root to vegetative shoot biomass, reproductive effort (ratio biomass of mature spikelets to the total biomass), abortion rate (percentage of aborted spikelets to the number of initiated spikelets).

### DATA ANALYSIS

A 'contextual analysis' was used to estimate multilevel selection on correlated characters with multilevel selection defined as the variation in individual fitness due to both properties of the individuals and properties of a group to which they are members (Heisler & Damuth, 1987; Damuth & Heisler, 1988). In this analysis, a selection-gradient method (Lande & Arnold, 1983) incorporates the effects from subdivision of a population into nested units. Technically this is completed by incorporating both individual and group characters into a multiple regression as independent variables and estimating their relative strength as predictors of an individual fitness measure. 'Aggregate characters' (*sensu* Heisler & Damuth, 1987), i.e. components of individual's phenotype created by combining measurements made on individuals, were used as contextual, i.e. shared by all members of a group, characters.

We combined the contextual analysis with path analysis following Stevens *et al.* (1995). A complex relationship between multiple traits and individual fitness was portrayed in an explicit system of linear causation, incorporating the effects of both measured and unmeasured traits on fitness (Crespi & Bookstein, 1989). A principal component analysis, followed by rotation (Varimax method, eigenvalue >1.0 as

minimum extraction parameter) yielded four major rotated factors, which were biologically meaningful and could be identified as major unmeasured traits (Table 1). The first three rotated factors (RF) were found to be normally distributed, and their within-group (i.e. ecotype) means (RF) used in a multiple regression on each of two fitness components, yield (number of mature spikelets) and reproductive biomass (total weight of mature spikelets). The regression model was

$$W_{kj} - W_{..} = \beta_{RF1}(z_{1kj} - z_{1..}) + \beta_{RF2}(z_{2kj} - z_{2..}) + \beta_{RF3}(z_{3kj} - z_{3..}) + \beta_{RF1}(z_{1.j} - z_{1..}) + \beta_{RF2}(z_{2.j} - z_{2..}) + \beta_{RF3}(z_{3.j} - z_{3..}) + \epsilon_{kj}$$

where  $W_{kj}$  is the fitness of the  $k$ th individual of ecotype  $j$ ;  $W_{..}$  is the mean fitness;  $z_{ikj}$  is the value of  $i$ th rotated factor measured on the  $k$ th individual of ecotype  $j$ ;  $z_{i.j}$  is the mean value for the  $i$ th rotated factor for the ecotype  $j$ ; and  $\epsilon_{kj}$  is the random error term for the  $k$ th individual of ecotype  $j$ . The individual-level coefficients  $\beta_{RF1-3}$  are the partial regression coefficients of fitness on each RF, controlling for ecotype and other individual-level factors, and the ecotype-level coefficients  $\beta_{RF1-3}$  are the partial regression coefficients of fitness on each ecotype  $RF$ , controlling for individual- and other ecotype-level factors. If a partial regression coefficient of fitness on the ecotype mean of a factor  $\beta_{RF}$  does not differ significantly from zero, the contextual character (ecotype identity) has no effect on

individual fitness. If, however, the ecotype regression coefficient is significantly non-zero, ecotype identity defines individual fitness together with individual phenotypic differences, and, in the case of their negative relationship, may indicate antagonism between the past selection in the environment of origin and experimentally induced selection under manipulated conditions. The latter is most likely to emerge from various trade-offs as a result of specialization to one environment constraining good performance in another environment.

The statistical analysis was done for each treatment separately using SYSTAT version 7.0 (SYSTAT, 1997).

RESULTS

The rotated factor analysis identified four major unmeasured factors that together accounted for 67.7% of the variance in the measured individual traits (Table 1): ‘plant size’ (RF1), ‘size of reproductive structures’ (RF2), ‘transition to reproduction’ (RF3) and ‘size of leaves’ (RF4). The first three unmeasured factors are of great importance in determining individual performance and are involved in local adaptation of wild barley (Volis, 2001; Volis *et al.*, 2002).

Under favourable conditions (high water and nutrients, HH) both individual and ecotype plant size (RF1) were significant predictors of individual plant fitness, either estimated by reproductive biomass or yield

**Table 1.** Rotated Factors’ (RF) loadings for 20 measured individual traits. Major factor loadings in bold

Trait	RF1	RF2	RF3	RF4	RF5	RF6
Total biomass	<b>0.941</b>	0.191	0.06	0.24	-0.06	-0.02
Above-ground biomass	<b>0.917</b>	0.174	0.09	0.27	-0.15	-0.03
Shoot biomass	<b>0.877</b>	0.028	0.04	0.25	-0.31	0.06
Number of tillers	<b>0.873</b>	0.013	0.15	0.18	0.12	0.04
Root biomass	<b>0.723</b>	0.203	-0.08	0.02	0.42	0.01
Spike length	0.254	<b>0.874</b>	0.00	0.08	0.03	-0.08
Spikelet weight (mg)	0.027	<b>0.867</b>	-0.19	-0.08	0.09	-0.11
Tiller height	0.254	<b>0.775</b>	0.14	0.19	0.20	-0.02
Number of nodes	0.035	<b>0.558</b>	-0.41	-0.12	-0.03	0.02
Awn length	-0.070	<b>0.548</b>	0.43	0.07	-0.16	0.09
Days to anthesis	-0.080	0.076	<b>-0.91</b>	-0.24	0.12	0.11
Days to awn appearance	-0.110	0.039	<b>-0.89</b>	-0.24	0.11	0.15
Spikelets per spike	0.436	0.123	0.54	0.50	0.17	-0.11
Flag leaf width	0.184	0.056	0.21	<b>0.86</b>	-0.10	-0.04
Penultimate leaf length	0.245	-0.03	0.18	<b>0.86</b>	0.12	0.02
Flag leaf length	0.246	-0.072	0.21	<b>0.86</b>	0.04	0.06
Penultimate leaf width	0.105	0.128	0.05	<b>0.76</b>	-0.18	-0.07
Root:shoot biomass	-0.09	0.101	-0.17	-0.08	<b>0.86</b>	0.07
Abortion rate	0.075	-0.04	-0.13	0.00	0.11	<b>0.93</b>
Reproductive effort	0.121	0.493	0.27	0.15	0.39	-0.52
Variance explained (%)	21.60	15.70	13.00	17.40	7.00	6.10

**Table 2.** Partial regression coefficients of fitness estimates (reproductive biomass and yield) on individual and group RFs under each of three treatments. Asterisks denote that slopes are significantly different from slope of zero

Traits	HH		HL		LH	
	Repro. biomass	Yield	Repro. biomass	Yield	Repro. biomass	Yield
RF1 (Plant size)	0.32***	0.30***	0.34***	0.27***	0.87***	0.86***
RF2 (Size of reproductive organs)	0.38***	0.12	0.20***	-0.08	0.70***	0.11
RF3 (Transition to reproduction)	0.04	0.10	0.03	0.06	0.19	0.36***
<i>RF1</i>	-6.42***	-4.59*	-0.14	1.16	0.60	-0.82
<i>RF2</i>	-0.26*	-0.32*	-0.07	0.27	-0.22	-0.45
<i>RF3</i>	-2.05***	-1.38*	-0.07	1.22	0.30	0.23

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

(Table 2). Both individual and ecotype size of reproductive structures (reproductive tillers, spikes and spikelets) (RF2) was significantly related to individual reproductive biomass, however, individual yield significantly correlated with only ecotype RF2. Transition to reproduction (RF3) correlated with neither reproductive biomass nor yield at individual level, but correlated with both estimates of fitness at ecotype level (Table 2). In all cases selection at the individual and ecotype levels was in opposite directions (Fig. 1). It should be noted, that because the major loadings for RF3 (days to anthesis and awn appearance) were negative, the positive correlation of RF3 with fitness indicates selection for early onset of reproduction and the negative relationship denotes that delay in reproduction is selected for.

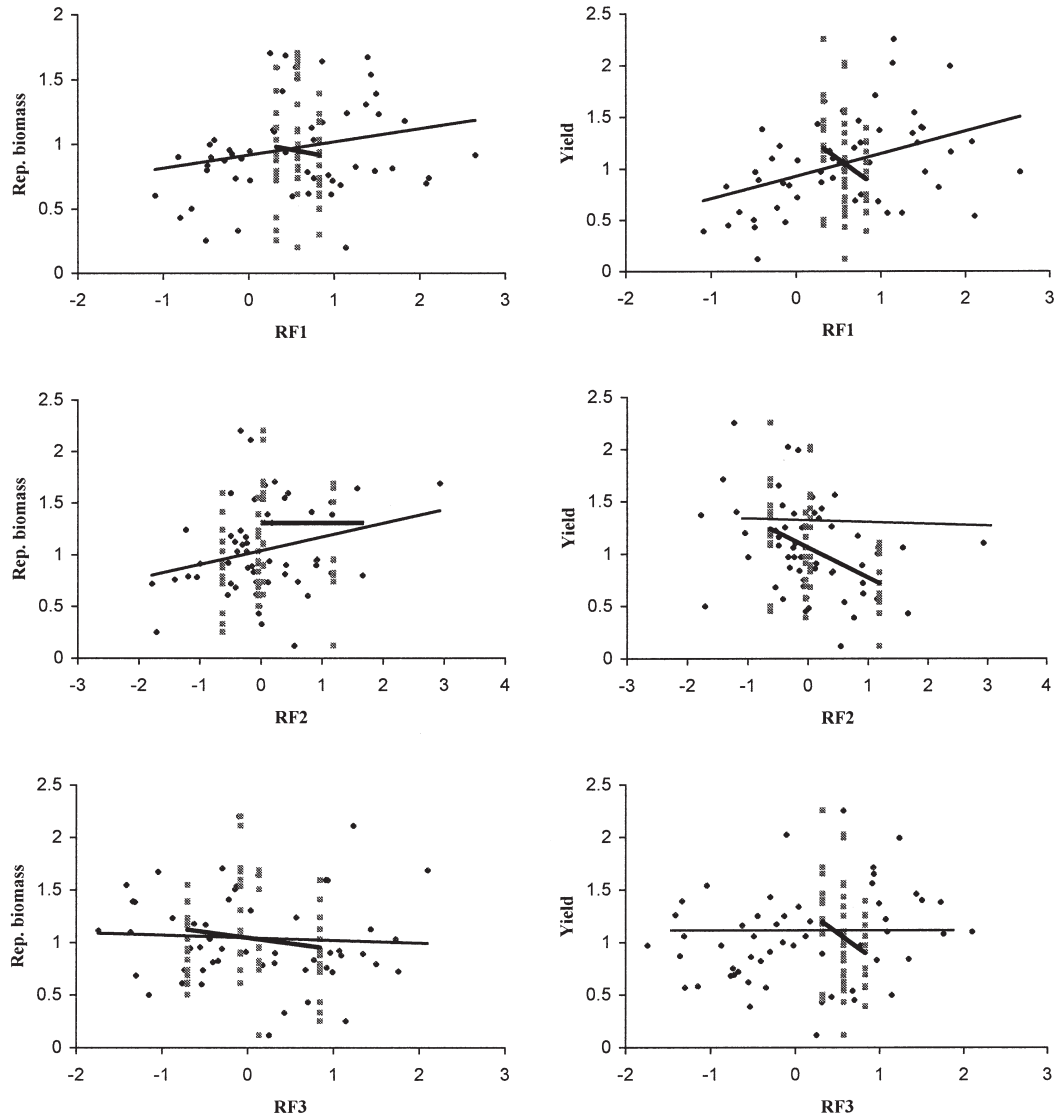
Under less favourable conditions, either low water high nutrients (LH) or high water low nutrients (HL) the regression of reproductive biomass on RF1 and RF2 and of yield on RF1 was significant only at individual and not ecotype level (Table 2). The response of plants to the LL was found not to differ from the response to LH and was omitted.

## DISCUSSION

We found a significant impact of plant origin (ecotype identity) on plant individual fitness under favourable experimental conditions (high availability of water and nutrients). No effect of ecotype identity on individual fitness was detected under conditions of stress (low availability of either water or nutrients). Individual selection favoured increased size of plants and reproductive structures and advanced transition to reproduction, while apparent group (ecotype) selection was in the opposite direction. We are to interpret the observed effect of ecotype identity on individual fitness as not a current group selection but as a constraining

effect of ecotype specific past selection. The four ecotypes went through environmentally specific selection process in their own environments with the optimal strategy evolved; consequently, this strategy may have a constraining effect on plant performance in other environments. We previously reported (Volis, 2001) that inversely related environmental productivity and unpredictability appears to induce two alternative evolutionary pathways that evolve seed size and number of seeds produced. The desert (SB) and grassland (AM) ecotypes were found to represent opposite choices *per se* (high number of small seeds vs. few large seeds, respectively) and semisteppe batha (BG) ecotype to compromise the alternatives. Onset of reproduction is also a part of optimal strategy and unproductive/unpredictable vs. productive/predictable environment selects for early vs. late onset of reproduction. The desert ecotype was the earliest and the mountain (MH) ecotype was the latest in start of reproduction (Table 3). The observed discrepancy between individual and ecotype selection may reflect an ecotype specific pattern of traits' relationship with each other and with fitness components. A formal selection gradient analysis done for each ecotype separately would reveal the causal mechanism of ecotype specific responses under favourable conditions, but it requires a large sample size and can not be conducted on the data used in this study.

No effect of ecotype identity on individual fitness was observed under less favourable conditions (low availability of either water or nutrients). This may suggest a similar mechanism for plant responses to stress that is largely independent of plant origin, with a difference between ecotypes under stressful conditions due entirely to difference in amount but not architecture of plasticity. Analysis of overall ecotype plasticity in response to water and nutrient stresses reported in a companion paper (Volis *et al.*, 2002)



**Figure 1.** The relationship of plant size (RF1), size of reproductive organs (RF2) and transition to reproduction (RF3) with individual (diamonds) and ecotype (solid squares) fitness components (reproductive biomass and yield) under high water high nutrients treatment.

supports this hypothesis. Also in a study of Zhang & Lechowicz (1994) the amount of overall plasticity among populations of *Arabidopsis thaliana* in response to nutrient stress was different but the pattern of plasticity remained similar.

Our study reports a first application of contextual analysis to detect ecotype specific past selection in hierarchically structured populations. All up-to-date studies used contextual analysis to detect a current group selection that may arise from altruism and frequency-dependence (Goodnight *et al.*, 1992; Stevens *et al.*, 1995; Tsuji, 1995; Bansbach & Herbers, 1996). Our study using methodology proposed by Heisler &

Damuth (1987) in modification of Stevens *et al.* (1995) shows that contextual analysis can be used for detection of selection at above-individual level without necessity to assume group selection. When populations are hierarchically structured, and environmental conditions vary along locations, the ecotypes are likely to undergo different local selection regimes; consequently these past selection effects may oppose current selection when individuals are introduced into other environments. The procedure of multilevel selection analysis in its simplest form requires: (1) a common garden design with measurements of both phenotypic traits and estimates of fitness; and (2) a

**Table 3.** Comparison of ecotype means for traits contributed to the first three rotated factors under high water high nutrients treatment by Fisher's LSD method after 1-way ANOVA. Letter order is from highest to lowest for all traits

Trait	F	Populations			
		SB	BG	AM	MH
Total biomass	0.4 ns	–	–	–	–
Above-ground biomass	1.0 ns	–	–	–	–
Shoot biomass	0.3 ns	–	–	–	–
Number of tillers	0.6 ns	–	–	–	–
Root biomass	8.1***	c	b	bc	a
Spike length	19.0***	c	b	a	b
Spikelet weight (mg)	22.8***	c	b	a	b
Tiller height	12.8***	bc	c	a	b
Number of nodes	8.1***	b	a	a	a
Awn length	2.0 ns	–	–	–	–
Days to anthesis	22.6***	d	c	b	a
Days to awn appearance	22.0***	d	c	b	a

\*\*\* $P < 0.001$ ; ns, not significant.

gradient-selection or path analysis where both individual and ecotype traits are included in a model.

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