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Habitat-specific natural selection at a flowering-time QTL is a main driver of local adaptation in two wild barley populations

K. J. F. VERHOEVEN,* H. POORTER,† E. NEVO‡ and A. BIERE*
*Center for Terrestrial Ecology, Netherlands Institute of Ecology NIOO-KNAW, PO Box 40, 6666 ZG Heteren, The Netherlands; †Plant Ecophysiology, Utrecht University, PO Box 800.84, 3508 TB Utrecht, The Netherlands; ‡Institute of Evolution, Haifa University, Mt Carmel, IL-31905 Haifa, Israel

Abstract

Understanding the genetic basis of local adaptation requires insight in the fitness effects of individual loci under natural field conditions. While rapid progress is made in the search for genes that control differences between plant populations, it is typically unknown whether the genes under study are in fact key targets of habitat-specific natural selection. Using a quantitative trait loci (QTL) approach, we show that a QTL associated with flowering-time variation between two locally adapted wild barley populations is an important determinant of fitness in one, but not in the other population's native habitat. The QTL mapped to the same position as a habitat-specific QTL for field fitness that affected plant reproductive output in only one of the parental habitats, indicating that the genomic region is under differential selection between the native habitats. Consistent with the QTL results, phenotypic selection of flowering time differed between the two environments, whereas other traits (growth rate and seed weight) were under selection but experienced no habitat-specific differential selection. This implies the flowering-time QTL as a driver of adaptive population divergence. Our results from phenotypic selection and QTL analysis are consistent with local adaptation without genetic trade-offs in performance across environments, i.e. without alleles or traits having opposing fitness effects in contrasting environments.

Keywords: local adaptation, antagonistic pleiotropy, flowering time, QTL analysis, reciprocal transplant, trade-offs

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Introduction

Local adaptation of plant populations to different environments plays an important role in the maintenance of intraspecific genetic variation (Gillespie & Turelli 1989) and in speciation (Schluter 2001). While local adaptation itself is readily demonstrated in reciprocal transplant experiments (Kawecki & Ebert 2004), basic questions concerning the genes and genetic mechanisms that underlie adaptive differentiation between populations remain largely unanswered (Orr 2005; Phillips 2005). A fundamental step is to identify the genes that are targets of natural selection under native field conditions, and importantly, those that are key targets of differential selection between habitats. In search of such genes, the genetic basis of variation in ecological traits between populations or ecotypes has been dissected in several model and nonmodel plant species (e.g. Slate 2005; Mitchell-Olds *et al*. 2007; Shindo *et al*. 2007).

In order to identify loci that play a key role in local adaptation, it is essential that fitness consequences of observed genetic differences are assessed in the native field environments in which the genetic variation evolved (Mitchell-Olds & Schmitt 2006; Mitchell-Olds *et al*. 2007; Shindo *et al*. 2007). While several exciting field transplant studies have been performed that assess fitness effects of allelic variants at specific candidate genes or quantitative trait loci (QTL) (Lexer *et al*. 2003; Weinig *et al*. 2003; Korves *et al*. 2007), only few studies have done so using reciprocal transplants between contrasting field habitats that are native to the

Correspondence: Koen J. F. Verhoeven, Fax: +31-26-4723227; E-mail: k.verhoeven@nioo.knaw.nl

genotypes under study (Verhoeven *et al*. 2004b; Gardner & Latta 2006). Moreover, to date, these efforts have not identified loci for specific functional traits as important targets of differential selection between the native habitats. Such experiments are now feasible in several ecological model species, and have recently been characterized as the 'low-hanging fruit' in this area of research (Mitchell-Olds *et al*. 2007). Here we present such a study, showing that a flowering-time locus is an important determinant of local adaptation of two wild barley populations from contrasting habitats.

Insight into the genetic mechanisms underlying local adaptation is necessary to understand and predict opportunities of plant species to adequately respond to changing environments. Phenotypic selection is imposed on traits that can have complex genetic architectures, including interacting genes, genes that affect multiple different traits (pleiotropy), and environment-specific gene effects. This architecture affects opportunities for adaptive evolution and divergence. For instance, pleiotropy and also close genetic linkage are causes of trait correlations that may hinder the independent evolution of individual traits (Gardner & Latta 2007). Adaptive differentiation in a heterogeneous environment, on the other hand, may be facilitated if selection targets the same alleles in different environments but in opposite directions. If there are individual genes (alleles) that cause above-average fitness in one environment but, at the same time, below-average fitness in another environment, there can be no single recombinant genotype that is best in both environments. Such genetic trade-offs are often assumed in theoretical models of ecological specialization (see Fry 1996; Kawecki & Ebert 2004), and they have been demonstrated empirically in some systems (Hawthorne & Via 2001).

In search for the genes that control variation in ecologically relevant traits, QTL analysis based on interpopulation crosses is often used as a first step to pinpoint the genomic regions responsible for trait variation (e.g. Erickson *et al*. 2004; Koornneef *et al*. 2004; Slate 2005; Bratteler *et al*. 2006). QTL analysis provides low-resolution genetic information but has the advantage of providing a genome-wide context for evaluating the phenotypic effects of individual genome regions, thus identifying the most important regions that control trait variation between the genotypes under study. By transplanting replicated QTL recombinant lines into natural field environments the fitness consequences of allelic variation at individual genomic regions can be assessed (Lexer *et al*. 2003; Weinig *et al*. 2003; Verhoeven *et al*. 2004b; Gardner & Latta 2006). This results in a global picture of the important loci that are targeted by natural selection and permits an evaluation of the role of genetic constraints and trade-offs in adaptive evolution. The subsequent identification of the underlying causal gene or genes within a QTL domain requires higher-resolution approaches such as candidate gene analysis (Kroymann & Mitchell-Olds 2005). However, without a genome-wide context as provided by QTL analysis the relative importance of individual candidate genes remains unclear.

We used a QTL approach to investigate the genetic basis of local adaptation in two populations of wild barley (*Hordeum spontaneum* Koch), an annual and mostly selfing grass from the Middle East (Nevo *et al*. 1979) that is the progenitor of cultivated barley. We mapped QTL for several life-history traits, and linked this information to available field fitness scores of the same QTL lines from a previously published field transplant experiment conducted in the two native habitats of the parental genotypes (Verhoeven *et al*. 2004b). This allowed us to explore patterns of differential natural selection on traits and QTL between habitats. We show that a QTL responsible for flowering-time variation between the populations is a main determinant of adaptive population divergence. Loci for two other important life-history traits, relative growth rate (RGR) and seed weight, are under selection as well, but not under differential selection in the two habitats; hence, variation at these loci does not contribute to local adaptation. Our results are consistent with adaptive divergence of the populations in the absence of genetic trade-offs: we observed no alleles or traits with opposite fitness effects in the contrasting natural environments.

Materials and methods

Plant material

Wild barley, *Hordeum spontaneum*, occurs in various habitats in the Near East (Nevo *et al*. 1979). We studied a coastal Mediterranean population [near Ashqelon (AQ)] and an inland steppe population [near Mehola (ME)] from Israel. Both sites have a rain-limited growing season, which is shorter and less predictable in the alluvial, rich steppe environment than in the sandy, poor coastal environment. Seeds of the two populations were obtained from the collection at the Institute of Evolution, Haifa University, Israel. Site descriptions and evidence for local adaptation from a standard reciprocal transplant experiment are presented elsewhere (Verhoeven *et al*. 2004b). An F_2 mapping population was derived from an interpopulation cross and genotyped at amplified fragment length polymorphism and simple sequence repeat markers as described in Poorter *et al*. (2005); these markers were used to construct a linkage map (Poorter *et al*. 2005) and for QTL mapping. The linkage map was estimated to cover approximately half of the genome. Under greenhouse conditions, 140 F_2 plants were selfed to generate 140 F_3 seed families ('F_3 lines'), which were used in phenotyping experiments in addition to the parents and five additional natural accessions from each of the populations.

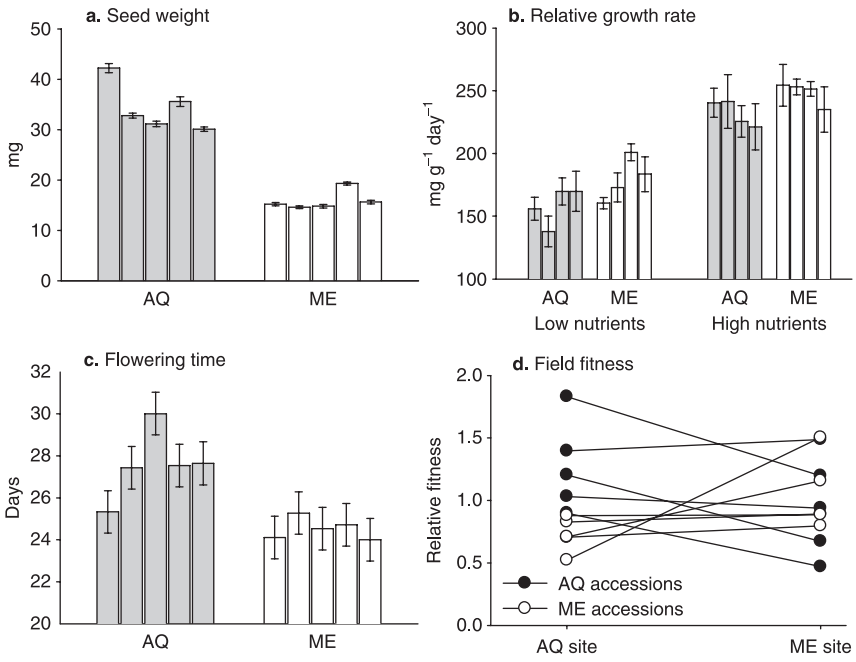

Fig. 1 Phenotypic differences between Ashqelon and Mehola accessions. (a–c) Life-history traits (mean ± SE) measured in controlled environments. Closed bars are Ashqelon (AQ) accessions and open bars are Mehola (ME) accessions. Growth rate data are from Verhoeven *et al*. (2004a). (d) Line mean estimates of relative fitness (within-site mean = 1) for plants transplanted into the native AQ and ME field sites. Field fitness data are from Verhoeven *et al*. (2004b).

Fitness scores and trait measurements

Fitness scores of the F_3 lines and accessions at the natural field sites were taken from Verhoeven *et al*. (2004b) who performed a transplant field experiment in the native AQ and ME environments. Briefly, at each site 30 seeds per line and accession were planted in experimental plots among natural vegetation, and plots were left undisturbed during the growing season. Germination and survival until reproduction were scored in all plants. Number of tillers, seeds per tiller, proportion of fertile seeds, and mean individual seed weight were measured in subsets of available plants, and least squares line means were calculated in linear models that accounted for various sources of nongenetic variation (plots, harvests); see Verhoeven *et al*. (2004b) for details. Germination was not affected by seed weight at either site (data not shown), and as nearly all germinated plants survived to produce seeds we decided to restrict our analysis to reproductive fitness of reproducing plants. We used total seed biomass output of reproducing plants as a fitness estimate, and calculated this for each F_3 line and accession as the product of the line means of the underlying component fecundity traits (tillers × seeds per tiller × proportion fertile seeds × mean individual seed weight).

Trait measurements of F_3 lines, parental genotypes and accessions from both parental populations were obtained in different experiments carried out under greenhouse, climate chamber and experimental garden conditions (see below). We focused on three life-history traits that we speculated to be relevant for fitness in the investigated field environments that differed in fertility and length of the growing season: seed weight, RGR and flowering time (see, for instance, Aronson *et al*. 1992; Volis *et al*. 2002; Poorter & Garnier 2007).

Seed weight. Upon greenhouse propagation of the F_2 plants, and prior to outplanting the seeds in the field experiment described above, seeds were dried at 40 °C and kernels were weighed in batches of six (n = 10 batches per F_3 line and accession; for Fig. 1, mean values were divided by six to estimate individual seed weights).

Flowering time. Flowering time was measured at different nutrient levels in an experimental garden at the Netherlands Institute of Ecology, Heteren, the Netherlands, in spring 2001 (two nutrient levels, 140 F_3 lines and 10 accessions, n = 8 individuals per F_3 line and accession). Experimental conditions are described in Verhoeven et al. (2004b). Briefly, young seedlings were vernalized (3–4 weeks at 5 °C), transferred individually to 3-L pots and placed outside. Pots were watered several times per day via drip-irrigation. Nutrient levels were applied by mixing different concentrations of a slow-release fertilizer through the potting soil. A standardized competition treatment was imposed by adding two *Avena sterilis* seedlings to each pot. Plants were checked every other day for signs of flowering (visible emergence of awns from the first tiller). Flowering time, or the number of days between transplanting seedlings to outside pots and visible awn emergence, was unaffected by nutrient treatment [mean F_3 flowering time: 24.5 days at high nutrients and 24.7 days at low nutrients; nutrient

effect $F_{1,7} = 3.2$ (not significant); nutrient × line effect $F_{139,920} = 0.9$ (not significant); linear mixed model using block as random factor and nutrient and genotype as fixed factors], and nutrient levels were therefore pooled to calculate F_3 line mean scores.

Relative growth rate. RGR scores for accessions and F_3 lines were taken from two separate experiments described in Verhoeven *et al*. (2004a) and Poorter *et al*. (2005). Seedling growth of the 10 accessions was determined for pot-grown plants at different nutrient levels under greenhouse conditions in an experiment that included the AQ and ME accessions plus several other *H. spontaneum* populations, and that is published elsewhere (Verhoeven *et al*. 2004a). Classical growth analysis was performed based on dry weights on days 17–26 after germination and using random pairing of plants across the harvest interval ($n = 4$ pairs). RGR scores of the F_3 lines are taken from Poorter *et al*. (2005), who calculated relative growth rates based on repeated measurements of total plant fresh weight in hydroponically grown plants, weighed individually on days 14 and 21 after germination ($n = 5$).

Data analysis

All analyses (except QTL analysis) were performed in SAS version 9.1 for Windows (SAS Institute). We fitted linear mixed models to test for population differences (using accession data) in seed weight, RGR and flowering time; flowering data were log-transformed to improve normality. For seed weight, factors included in the model were population and accession. For RGR and flowering time, factors included were population, accession, block and nutrients. Accessions were nested within populations. Main block effects and two-way interactions involving blocks were included in the models but higher block interactions were pooled in the error term. Nutrients and population were considered fixed effects, all other terms random. For RGR, the difference between population means was tested via the AQ–ME contrast in a data set that also included other populations (Verhoeven *et al*. 2004a).

Phenotypic selection regimes at the AQ and ME sites were characterized by assessing how differences in the life-history traits among the F_3 lines (measured under controlled conditions) affected their fitness under field conditions. The mapping population provides a suitable tool for selection analysis because recombination and segregation in the cross break up trait associations present in parental genotypes, thus facilitating the estimation of independent fitness effects of individual traits. Using line means, we regressed relative fitness (total seed biomass output divided by the mean seed biomass output of all lines in that environment) on individual traits to estimate total selection, while multiple regression on all traits jointly yielded estimates of direct selection in order to identify targets of selection (Lande & Arnold 1983). Note that our approach of regressing line mean field fitness scores on line mean scores of functional traits measured in controlled environments assumes reasonable correspondence in F_3 line rank scores for traits in the controlled environment and field experiments, which is an untested assumption. The approach reduces bias by minimizing the contribution of within-site environmental heterogeneity to trait-fitness covariances (Mauricio & Mojonnier 1997). All three predictor traits where standardized (mean = 0, SD = 1) so that regression coefficients represent standardized selection gradients (Lande & Arnold 1983). We checked for stabilizing or disruptive selection via regression effects of quadratic trait scores on fitness over and above the traits' linear effects (Lande & Arnold 1983) but found no evidence for significant nonlinear selection. Differential selection between the sites was tested via the trait × site interaction effect on fitness in linear models that included site as a fixed effect and single traits (differential total selection) or all traits simultaneously (differential direct selection) as covariates.

QTL analyses were performed using the F_2 design in MAPQTL (Van Ooijen & Maliepaard 1996) by taking the F_3 line mean values as estimates for the trait value of their respective F_2 parents. For each trait, an initial interval mapping genome scan was carried out, followed by repeated composite interval mapping with markers near previously detected LOD peaks included as cofactors until LOD profiles stabilized. We report LOD peaks exceeding 2.8; this threshold ensures a chromosome-wide type I error rate of < 0.05 for the wild barley map (Van Ooijen 1999) and can be considered suggestive of QTL presence. We evaluated colocation of QTL as overlapping 1-LOD or 2-LOD drop support intervals, the latter corresponding approximately to a 95% confidence interval for LOD peak location (Van Ooijen 1992). Using the above procedure, QTL for RGR and field fitness were published previously (Verhoeven *et al*. 2004b; Poorter *et al*. 2005); in this study, additional QTL for flowering time and seed weight are presented. Fitness QTL that appeared environment-specific (that is, were significant in only one of the field environments) were subjected to an additional analysis that included observations from both field sites to formally test for environment-specificity of the QTL effect. For individual markers within such QTL domains, linear models were fitted that included site, marker and their interaction; a significant interaction term indicates that the marker effect differs between environments. As absolute fitness scores differed greatly between the AQ and ME sites, this analysis necessitated the use of unequal-variances models that estimated different error variances for each site, in order not to violate standard ANOVA assumptions (available in PROC MIXED in SAS).

Table 1 Natural selection gradients for life-history traits in native field environments. Direct and total selection gradients that are significantly different between sites are printed in boldface. Growth rate data are from Poorter *et al*. (2005) and traits are linked to field fitness data from Verhoeven *et al*. (2004b)

	Selection	
	Direct	Total
AQ site		
Seed weight	0.11***	0.10***
Relative growth rate	0.04*	0.01
Flowering time	**–0.04***	**–0.05***
ME site		
Seed weight	0.10***	0.09***
Relative growth rate	0.06*	0.04
Flowering time	**–0.13*****	**–0.14*****

*$P < 0.05$, ***$P < 0.001$.

Results

Population differences

The AQ and ME populations differed significantly in all life-history traits measured: under controlled conditions, inland ME plants had smaller seeds (population effect: $F_{1,8} = 63$, $P < 0.001$), higher RGR (contrast AQ–ME populations: $F_{1,24} = 4.5$, $P < 0.05$), and they flowered earlier (population effect: $F_{1,7} = 16.9$, $P < 0.01$) than coastal AQ plants (Fig. 1a–c). Local adaptation of the populations, as demonstrated previously in a reciprocal transplant experiment (Verhoeven *et al*. 2004b), is visualized in Fig. 1d: at the AQ site, the native (AQ) accessions outperformed the non-native (ME) accessions, while at the ME site all native (ME) accessions outperformed at least some of the non-native (AQ) accessions.

Phenotypic selection regimes in the native habitats

Variation in the three life-history traits explained 33% of fitness variation at the ME site and 26% at the AQ site (model r^2 of within-site multiple regressions of fitness on the three life-history traits). Similar targets of natural selection were revealed at both sites: large seeds, high RGR and early flowering conferred direct fitness benefits at both field sites (Table 1). But the strength of selection differed between sites: flowering time was targeted more strongly at ME than at AQ (flowering time × site interaction: $F_{1,272} = 12$, $P < 0.001$). No differential selection between sites was observed for seed weight and RGR. At the ME site, flowering time had the strongest effect on fitness, while at the AQ site seed weight had the strongest effect.

Across the F_3 lines, seed weight was negatively correlated with RGR ($r = -0.31$, $P < 0.001$). Both traits experienced positive selection, but this correlation prevents fitness maximization via both traits simultaneously. Total selection of these traits was thus lower than direct selection (causing the RGR total selection gradient to drop below the significance threshold; Table 1) as a direct positive fitness effect of one trait is counteracted by a correlated negative effect via selection on the other trait.

QTL analysis

The two parental genotypes carried beneficial alleles at QTL for those traits that are locally most important: increased seed weight was associated with AQ alleles and early flowering was associated with ME alleles at the respective QTL (Fig. 2). Consistent with the observed correlation between seed weight and RGR, QTL for these traits mapped to the same position on linkage group 5(1H) with allelic effects in opposite direction. AQ alleles reduced RGR but increased seed weight, and vice versa for ME alleles.

Aligning the life-history QTL with QTL for field fitness, we explored patterns of QTL overlap (Fig. 2). Field fitness QTL represent targets of natural selection at the genetic level, and QTL that control phenotypic traits under selection are expected to colocate with fitness QTL (Lexer *et al*. 2003; Ungerer & Rieseberg 2003). Fitness QTL colocated with QTL for RGR, seed weight and flowering time, and in all cases, the direction of allelic affects was in agreement with the phenotypic selection analysis. Site-specific fitness QTL reflect differential selection between sites, and point at loci that may underlie adaptive differentiation. Two fitness QTL were significant at one site only [at linkage groups 2b(2H) and 7b(5H), see Fig. 2]. One of these [at group 7b(5H)] showed convincing evidence of environment-specificity, as all analyzable markers within this domain showed significant marker × site interactions in a joint analysis across sites, with the strongest marker × site interaction observed for the marker at position 10 (see Fig. 2: $F_{1,53} = 11.5$, $P = 0.001$). The fitness QTL at group 2b(2H) showed mixed evidence of environment-specificity: one of the analyzable markers within the domain showed a significant marker × site interaction (marker at position 0: interaction $F_{2,110} = 3.4$, $P = 0.04$) but other markers in the domain did not.

A flowering-time QTL that accounted for 31% of the variation in line mean trait scores colocated with the environment-specific fitness QTL at group 7b(5H). The early flowering QTL allele was significantly associated with high fitness in its native inland environment (with strong phenotypic selection for early flowering) but not in the coastal environment (with relaxed selection on flowering time).

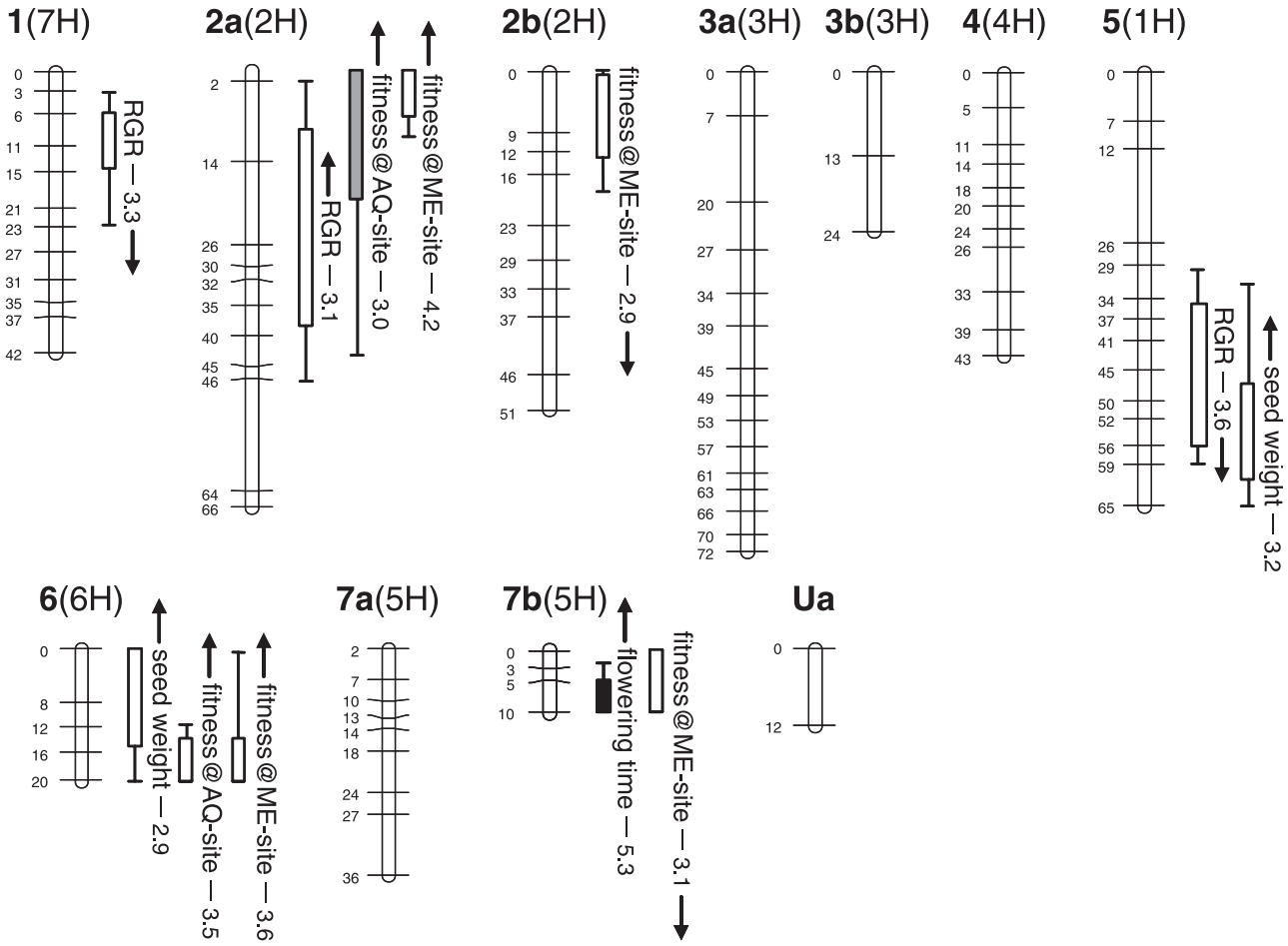

Fig. 2 Linkage map with QTL for life-history traits in controlled environments and for fitness in the native Ashqelon and Mehola field environments. Boxes and whiskers indicate 1-LOD and 2-LOD support intervals of peak location. Box shading denotes QTL effect size: white boxes < 15% explained variance of line means; grey boxes 15–25%; black boxes > 25%. Upward arrows indicate that Ashqelon (AQ) alleles increase the trait value and downward arrows indicate that Mehola (ME) alleles increase the trait value. For each QTL, the peak LOD score is given. Note that at linkage group 7b(5H), the AQ allele increases flowering time and therefore the ME allele causes early flowering. QTL for RGR and field fitness were published previously in Poorter *et al*. (2005) and Verhoeven *et al*. (2004b). The linkage groups from this map could be tentatively assigned to known *Hordeum* chromosomes based on shared markers with other published *Hordeum* maps, as described in Poorter *et al*. (2005; see also text). Chromosome names 1–7 refer to Triticeae syntenic map chromosomes, with corresponding barley chromosome names (1H–7H) added in parentheses.

Discussion

Differential natural selection at life-history QTL

Our study shows that adaptive divergence of the two wild barley populations is associated with habitat-specific differences in natural selection at a QTL that controls flowering-time variation. While most traits and QTL were targeted by selection similarly in both field environments, differential selection between environments was indicated for flowering time both at the trait level and at the QTL level. The colocation of a major flowering-time QTL with one (of only two observed) habitat-specific fitness QTL, and the agreement of its allelic effects with environment-specific phenotypic selection on flowering time, suggest that an underlying flowering-time gene within this QTL region is a key target of differential selection between the two habitats and that the populations have adaptively diverged at this gene in response to this selection. Given the limited resolution of QTL mapping, however, conclusive evidence that the same gene that affects flowering time also affects field fitness needs to come from candidate gene or fine-mapping approaches.

The QTL results agree well with evidence from previous field studies that phenotypic selection on flowering phenology is a principal cause of ecotypic variation in annual plants (Schemske 1984; Bennington & McGraw 1995; Etterson 2004; Weinig & Schmitt 2004; Griffith & Watson

2005). In wild barley, early reproduction in steppe populations could be an adaptation to the short and unpredictable growing seasons of those environments that necessitate rapid completion of the life cycle (Aronson *et al*. 1992). Our results provide a context for studies that aim to detect evidence of natural selection at specific flowering-time candidate genes (Toomajian *et al*. 2006; Korves *et al*. 2007) by demonstrating the relative importance in native field environments of the flowering-time locus compared to other (genome-wide) loci.

Flowering-time control in barley is mediated by responses to day-length and prolonged periods of cold (vernalization), and several genes in the photoperiod and vernalization pathways have been identified (Cockram *et al*. 2007; Trevaskis *et al*. 2007). Our flowering-time QTL contained one marker (E39M61-272, at position 3 of group 7b(5H), see Fig. 2) that mapped to barley chromosome 5H at position 122 cM in a recently published high-density barley consensus map (Marcel *et al*. 2007). From studies in cultivated barleys, this chromosome region is known to contain one of the major barley vernalization-response loci VRN-H1 (Laurie *et al*. 1995; von Zitzewitz *et al*. 2005; Szucs *et al*. 2006). The *HvBM5a* candidate gene that has been demonstrated to underlie the VRN-H1 locus effect was recently mapped to position 124 cM of this chromosome on the barley consensus map (Cockram *et al*. 2008). This close correspondence in map positions of our flowering-time QTL with a gene that is known to play a central role in controlling flowering-time variation, via the vernalization response, in barley cultivars suggests that the same gene may be responsible for adaptive flowering-time evolution and divergence of the natural wild barley populations. This hypothesis needs to be tested by screening genetic variation at the VRN-H1 locus in a broader set of natural accessions, including more genotypes from the AQ and ME populations but also from other natural populations, and testing for associations with vernalization requirement and flowering time.

In this study, we measured RGR and flowering time under greenhouse or experimental garden conditions and tested the consequences of observed genetic differences in these traits for fitness under field conditions. This differs from traditional phenotypic selection analysis that uses trait and fitness scores from the same environment, and can only be interpreted as such to the extent that there is no genotype × environment (G×E) interaction for the life-history traits across the study environments. Absence of G×E interaction is unlikely. Nevertheless, limited available data for the F_3 lines from experiments in controlled environment show that G×E is weak at least with respect to some important environments: under contrasting nutrient regimes no significant G×E effects for flowering time (this study) and no significant G×E or environment-specific QTL were found for RGR (Elberse *et al*. 2004).

Divergence in the absence of genetic trade-offs?

Across the range of phenotypic variation that was generated by the crossing design, the three traits were selected in the same direction but, in the case of flowering time, at different intensities in the two field environments. Absence of traits or loci that are selected in opposite directions would imply that specialization to one habitat does not necessarily result in reduced fitness in the other habitat. While the number of traits explored in this study is too low to assess whether or not such opposite-effect traits play an important role in our study system, a previous analysis at the genetic level in the same system also showed that over large genomic areas, the fitness effect of individual loci often differed in the magnitude but not in the direction of their effects between the two environments (Verhoeven *et al*. 2004b). Together, the QTL and phenotypic selection results suggest that trade-offs might not play an important role in the adaptive divergence of the wild barley populations.

Adaptive differentiation is often thought to involve trade-offs in performance across environments, at least at some loci, because this precludes the existence of a recombinant genotype with highest fitness in all environments (Joshi & Thompson 1995). But without trade-offs, an overall pattern of local adaptation can still arise by the joint effects of several alleles that typically confer the larger fitness benefit at their home site or that only affect fitness in one environment, as demonstrated previously in theoretical studies (Fry 1996; Kawecki 1997; Kawecki *et al*. 1997). Differentiation without trade-offs could be promoted by severely limited levels of gene flow and recombination between diverging genotypes, that is characteristic of self-fertilizing species such as wild barley (Gardner & Latta 2006). This minimizes opportunities for recombinants to arise that combine beneficial alleles for both habitats. Interestingly, studies in self-fertilizing plants have been largely consistent with specialization in the absence of trade-off loci (Weinig *et al*. 2003; Latta *et al*. 2007; this study).

While our results are consistent with local adaptation without a cost of specialization, it is important to remain aware of the limitations of the experimental design of our study. For instance, in our QTL analysis, we used a genetic map that did not cover the entire genome (Poorter *et al*. 2005). The part of the genome that could be explored clearly showed that local adaptation in this system can be based on the joint effects of loci that differ in the magnitude but not direction of their fitness effect across environments. But the unexplored part, of course, could harbour trade-off loci. Also, while our field experiment covered one full growing season of the annual species from germination until reproduction, we have no information on selection pressures during the summer dormant phase of seeds. It is possible that environment-specific fitness effects of seed

weight exist in this phase (for instance, related to bet-hedging strategies of plants in unpredictable environments (Cohen 1966) that could favour the production of many small seeds) that were not captured in our experiment.

Within-environment constraints in the response to selection

Rapid growth and large seeds conferred selective advantages in both field environments, but a negative correlation between these traits prevents fitness maximization via both traits simultaneously. Our data illustrate the genetic basis of such a constraint by colocation of QTL for RGR and seed weight with opposing allelic effects. Although both traits were under selection under field conditions, this genomic region does not show up as a field fitness QTL probably because its fitness effect via one trait is offset by its effect via the other trait.

Conclusion

Our results demonstrate a selective advantage of native QTL alleles for the traits that are locally most important, and indicate a key role for a QTL that controls flowering time in adaptive differentiation of the populations. In addition, the results show that there is a within-environment pleiotropic constraint in the response to selection of individual traits (seed weight and RGR), and results are consistent with adaptive divergence of the populations in the absence of individual traits or loci that have opposite fitness effects in the different native environments. Based on candidate gene approaches, flowering-time genes have previously been suggested to play a role in adaptive differentiation of annual plants (Toomajian *et al*. 2006; Korves *et al*. 2007). The main contribution of our study is that, without a priori selecting such a locus, a flowering-time locus emerges in a genome-wide analysis as a key determinant of adaptive differentiation of the populations to their different native habitats.

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Koen Verhoeven is a postdoc at the Netherlands Institute of Ecology and a personal research grant holder of the Dutch Research Council. This paper follows from his PhD work on the genetics of local adapatation in wild Barley. He is currently working on epigenetic variation in asexually reproducing plants. Hendrick Poorter is a plant Ecophysiologist working on the mechanistic causes and ecological consequences of growth rate variation. Eviatar Nevo is a full Professor of Evolutionary Biology and Director of the Institute of Evolution, University of Haifa. Arjen Biere is an Evolutionary Ecologist with a main interest in plant-pathogen interactions.
