

## THE GENETIC BASIS OF ADAPTIVE POPULATION DIFFERENTIATION: A QUANTITATIVE TRAIT LOCUS ANALYSIS OF FITNESS TRAITS IN TWO WILD BARLEY POPULATIONS FROM CONTRASTING HABITATS

KOEN J. F. VERHOEVEN,<sup>1,2</sup> TYTTI K. VANHALA,<sup>3</sup> ARJEN BIJRE,<sup>1</sup> EVIATAR NEVO,<sup>4</sup> AND JOS M. M. VAN DAMME<sup>1</sup>

<sup>1</sup>Department of Plant Population Biology, Netherlands Institute of Ecology, NIOO-KNAW, P.O. Box 40, NL-6666 ZG, Heteren, The Netherlands

<sup>3</sup>Laboratory of Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, NL-6700 AJ, Wageningen, The Netherlands

<sup>4</sup>Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel

**Abstract.**—We used a quantitative trait locus (QTL) approach to study the genetic basis of population differentiation in wild barley, *Hordeum spontaneum*. Several ecotypes are recognized in this model species, and population genetic studies and reciprocal transplant experiments have indicated the role of local adaptation in shaping population differences. We derived a mapping population from a cross between a coastal Mediterranean population and a steppe inland population from Israel and assessed F<sub>3</sub> progeny fitness in the natural growing environments of the two parental populations. Dilution of the local gene pool, estimated as the proportion of native alleles at 96 marker loci in the recombinant lines, negatively affected fitness traits at both sites. QTLs for fitness traits tended to differ in the magnitude but not in the direction of their effects across sites, with beneficial alleles generally conferring a greater fitness advantage at their native site. Several QTLs showed fitness effects at one site only, but no opposite selection on individual QTLs was observed across the sites. In a common-garden experiment, we explored the hypothesis that the two populations have adapted to divergent nutrient availabilities. In the different nutrient environments of this experiment, but not under field conditions, fitness of the F<sub>3</sub> progeny lines increased with the number of heterozygous marker loci. Comparison of QTL-effects that underlie genotype  $\times$  nutrient interaction in the common-garden experiment and genotype  $\times$  site interaction in the field suggested that population differentiation at the field sites may have been driven by divergent nutrient availabilities to a limited extent. Also in this experiment no QTLs were observed with opposite fitness effects in contrasting environments. Our data are consistent with the view that adaptive differentiation can be based on selection on multiple traits changing gradually along ecological gradients. This can occur without QTLs showing opposite fitness effects in the different environments, that is, in the absence of genetic trade-offs in performance between environments.

**Key words.**—Antagonistic pleiotropy, fitness quantitative trait loci, genetic trade-off, heterosis, *Hordeum spontaneum*, local adaptation.

Received January 13, 2003. Accepted October 6, 2003.

Maintenance of genetic variation may be driven by selection for different genotypes in different environments (Hedrick 1986; Linhart and Grant 1996; Bell and Rebound 1997). Such local differentiation can lead to ecological specialization and is generally assumed to involve trade-offs between performance in contrasting environments that prevent one genotype from having maximal fitness in all environments (Hedrick 1986; Gillespie and Turelli 1989; Joshi and Thompson 1995). However, the genetic basis of adaptive differentiation is not well understood. If the same loci are under selection in contrasting habitats, with different alleles being favored in the two environments, then a true genetic trade-off exists: no genotype can be superior in both environments (Fry 1996). But the presence of loci with opposite fitness effects is not a necessary requirement for ecological specialization to evolve. Populations might also diverge if selection acts on different loci in different environments or if loci differ in the magnitude but not in the direction of their fitness effect across habitats (Fry 1996; Kawecki 1997), that is, in the absence of genetic trade-offs. Under both genetic scenarios locally adapted populations can evolve, with native genotypes outperforming nonnative genotypes. In plants, lo-

cal adaptation has often been demonstrated in reciprocal transplant experiments (e.g., Galen et al. 1991; van Tienderen and van der Toorn 1991; Jordan 1992; Bennington and McGraw 1995; Kindell et al. 1996; Nagy and Rice 1997), visualized by crossing reaction norms for fitness of genotypes transplanted into each other's native environment.

With the advance of quantitative trait locus (QTL) analysis it has become possible to identify chromosome regions responsible for quantitative trait variation (Lander and Botstein 1989) and to analyze effects of individual QTLs that underlie fitness variation. Several studies that have explored QTL effects of fitness-related traits across various environments have demonstrated a role of loci with opposite fitness effects across environments (Sari-Gorla et al. 1997; Jiang et al. 1999; Leips and Mackay 2000; Hawthorne and Via 2001). However, the majority of studies in agricultural crop species (e.g., Hayes et al. 1993; Lu et al. 1996; Courtois et al. 2000; Saranga et al. 2001; Teulat et al. 2001; Johnson and Gepts 2002; Xing et al. 2002) and natural species (e.g., Alonso-Blanco et al. 1998; Fry et al. 1998; Wu 1998) have typically revealed many loci that differ in the magnitude of their effect across environments but virtually no QTLs with opposite effects in different environments. Nearly all studies analyzed either nonnatural variation or tested the consequences of variation in environments in which parental genotypes had not evolved. Therefore, these studies give only limited insight into the

<sup>2</sup> Present address: Computational Genomics and Department of Agronomy, Purdue University, 915 West State Street, West Lafayette, Indiana 47907-2054; E-mail: kverhoeven@purdue.edu.

TABLE 1. Geographic (A), climatic (B) and edaphic/biotic (C) characteristics at the sites of origin of the Mehola and Ashqelon *H. spontaneum* populations, taken from Nevo et al. (1984). Additional data were collected during the experiment (D). Soil core samples were taken in each plot after the 1999–2000 growing season (particle sizes: clay [2  $\mu\text{m}$ ] < silt [53  $\mu\text{m}$ ] < sand; pH and available nutrients determined via  $\text{CaCl}_2$  extraction, also in 1998–1999 prior to growing season). Site productivity is indicated by the mean aboveground plant dry biomass clipped from 50  $\times$  50-cm areas adjacent to experimental plots.

	Ashqelon	Mehola
A. Elevation (m)	10	–150
Longitude	34.60	35.48
Latitude	31.63	32.13
B. Annual precipitation (mm)	424	270
January temperature ( $^{\circ}\text{C}$ )	14	13
Annual evaporation (cm)	120	180
Midday humidity (%)	64	34
C. Plant community	Park forest	Steppe
Soil type	Sandy loam	Alluvium
D. Precipitation 1999–2000 (mm)	331	226
Clay-silt-sand (%)	9-26-65	22-72-6
Soil pH	7.8	8.5
Organic matter (%)	2.5	4.9
Available P-K- $\text{NO}_3$ - $\text{NH}_4$ (mg/kg; 2000)	0.03-56-0.7-1.1	0.08-174-0.4-2.7
Available P-K- $\text{NO}_3$ - $\text{NH}_4$ (mg/kg; 1998)	0.03-40-1.4-4.5	0.35-247-2.0-26.3
Clipping biomass (g)	51	148

genetic basis of adaptive genotype  $\times$  environment interaction in natural populations (cf. Mitchell-Olds 1995b).

In the study presented here, we explored the effects of QTLs underlying fitness variation in the progeny of a cross between two contrasting natural populations of wild barley, *Hordeum spontaneum*, when transplanted into both natural environments of the parental genotypes. Crossing and subsequent recombination and segregation break up specific allele and trait combinations that are present in the parental genotypes. Analysis of the recombinant progeny therefore facilitates the estimation of independent fitness effects of individual alleles (or traits) and permits assessment of the genetic architecture of population differentiation. In addition to analyzing individual QTL effects, we assessed the fitness effects of genomewide heterozygosity and the genomewide proportion of native alleles. When crossing two locally adapted populations, progeny fitness under field conditions is expected to decrease (relative to the native parent) due to the disruption of coadapted gene complexes, either via dilution of the gene pool that was adapted to the local environment (Allard 1988; Waser and Price 1989) or via the disruption of intrinsic gene interactions that are unrelated to the local environment (Montalvo and Ellstrand 2001). This effect may be counteracted by heterosis, that is, the fitness advantage of heterozygosity that is often observed in the hybrid progeny of inbred line crosses, which is assumed to derive from either the masking of deleterious alleles or from heterozygosity at single loci conferring a greater fitness advantage than either homozygote (dominance vs. overdominance hypothesis; Charlesworth and Charlesworth 1987).

Here, we present the results of two experiments that were carried out to explore the genetic basis of adaptive population differentiation in two *H. spontaneum* populations. We conducted a field experiment in the natural habitats of the two populations where we reciprocally transplanted accessions to establish whether adaptive population differentiation has occurred. In the same experiment, we assessed fitness of the mapping population progeny lines to address the following

questions: Can we identify QTLs underlying fitness variation in the natural environments? To what extent do QTLs show interactions with the environment, and does selection favor native alleles over nonnative alleles at these loci? Is fitness in the recombinant  $F_3$  genotypes positively related to the genomewide proportion of heterozygous loci and to the proportion of native alleles? In a common-garden experiment, we tried to gain insight in the environmental causes of population differentiation. The environments of the populations under study differ in climatic, edaphic, and biotic characteristics (Table 1). Local adaptation, if it occurs, may be driven by any (combination of) these variables. Given a notable observed difference in habitat productivity that appeared unrelated to precipitation (Table 1), we explored soil nutrient status as an ecologically relevant variable to which the two populations may have differentially adapted. We tested progeny performance in a common-garden experiment in response to contrasting nutrient availabilities and asked whether QTLs underlying genotype  $\times$  nutrient interaction correspond to QTL effects observed in the field experiment. If divergent nutrient availability is indeed involved in adaptive differentiation of the populations at the field sites, then part of the QTL effects is expected to overlap.

## MATERIALS AND METHODS

### *Plant Material*

Wild barley, *H. spontaneum*, is a naturally selfing, diploid, annual grass with generally low levels of outcrossing (Brown et al. 1978) that grows in a range of habitats across the eastern Mediterranean basin and western Asia (Harlan and Zohary 1966). It is the progenitor of cultivated barley (Zohary 1969) and has been the focus of population, genetic, and agronomic studies across its ecological spectrum in the near east Fertile Crescent (for an overview, see Nevo 1992). Adaptive population differentiation in this species seems well established. Several ecotypes are recognized phenotypically (Snow and Brody 1984), and genetic population differentiation has been

shown to follow ecological gradients at macroscales (Nevo et al. 1986b) and microscales (Nevo et al. 1986a; Huang et al. 2002), which suggests adaptation to local environments. Differential adaptation to specific environmental variables has been demonstrated in common-garden designs (Lavie et al. 1994), and local adaptation has recently been evidenced in reciprocal transplant field experiments (Volis et al. 2002a,b). We studied *H. spontaneum* plants from a coastal population near Ashqelon (AQ) and a steppe inland population near Mehola (ME), Israel, representing respectively Savannoid-Mediterranean and Irano-Turanian phytogeographic communities (Danin 1988). Comparing the two sites, AQ shows slightly higher annual precipitation and humidity and lower evaporation, but also a coarser-grained soil type (i.e., poorer water retention) with less available nutrients (Table 1). A mapping population was derived from a cross between a plant from AQ and a plant from ME, as described in H. Poorter, C. P. E. van Rijn, T. K. Vanhala, K. J. F. Verhoeven, Y. E. M. de Jong, P. Stam, and H. Lambers (unpubl. ms.). The genotypes of F<sub>2</sub> plants were assessed using AFLP markers, and a molecular marker map was constructed (Poorter et al., unpubl. ms.). This map was estimated to cover 55% of the entire genome; due to unexpected heterozygosity of the AQ parent, some large linkage blocks (totaling roughly 45% of the genome) could not be mapped. From the mapping population, 140 plants were self-fertilized to yield 140 F<sub>2</sub>-derived F<sub>3</sub> families, which hereafter are referred to as F<sub>3</sub> lines.

#### Field Experiment

The experiment was carried out in Israel in the 1999–2000 winter growing season. Suitable experimental locations, that is, relatively undisturbed and with wild *H. spontaneum* growing nearby, were identified within a 5-km radius from the locations where the parental accessions had been collected. At each site, 3.5 × 5.5-m plots were prepared and planted with cleaned seeds (buried 2–3 cm deep, at regular intervals of 15 cm) prior to the onset of the growing season. Per site 30 seeds from each of 150 genotypes were planted: 140 F<sub>3</sub> lines, the parental accessions, and four additional accessions from both populations (randomized block design with six plots per site and five replicates per plot). Because annual rainfall at the experimental sites is rather unpredictable and in exceptional years is insufficient to sustain growth and flowering of *H. spontaneum* (pers. obs. in 1998–1999 season), half of the plots were irrigated once in the fourth week of December. At the ME site, seedlings of the large thistle *Silybum marianum* were removed from the plots by hand; plots were left undisturbed otherwise.

Germination was scored as the presence or absence of a live or dead seedling approximately one month after the onset of the season. Survival until reproduction, indicated by tiller and head (inflorescence) production, was scored at final harvesting. As seeds in *H. spontaneum* scatter from the plant upon maturation, we were unable to score all fecundity fitness traits (number of heads, seeds per head, and mean fertile seed weight) in all individual plants. Instead, we scored fecundity traits according to the following harvest design: each plot was divided in four quarters, and at four moments throughout the seed-ripening season one quarter of each plot was har-

vested. Measurements on the number of heads (reproducing tillers) were obtained at all four harvests. The number of seeds per head could be measured at the first two harvests only (counted in a maximum of five randomly chosen fully emerged heads). Mature seeds could be collected during the second and third harvest (randomly collected from available heads). The obtained data allowed for accurate estimation of line mean scores for fitness component traits, but power to analyze individual variation in total fecundity (total number or biomass of seeds produced) was limited because only a subset of individuals were scored for all fecundity component traits. The head count excluded the smallest and last-developed tillers that had elongated but showed no emerged inflorescence at the moment of harvesting, averaging 4.9% of tillers per plant at the ME site and 1.1% at the AQ site; these tillers generally do not produce fertile seeds as plants die due to terminal drought stress before these last heads can mature. Seeds were dried at 70°C for 48 h, checked for the presence of a kernel to distinguish aborted from fertile seeds, and mean seed weight was determined for the fertile seeds. These ‘‘seeds’’ are in fact entire dispersal units, and seed weights included lateral infertile spikelets and awns.

#### Common-Garden Experiment

This experiment was carried out at the NIOO research institute, Heteren, The Netherlands, in 2001. Cleaned seeds were germinated in petri dishes (4°C in darkness for 10–13 days), grown under greenhouse conditions for two days, and subsequently vernalized for three to four weeks (5°C, light/dark: 10/14 h, PAR 80–100 μmol m<sup>-2</sup> sec<sup>-1</sup>). Vernalized seedlings were transferred individually to 3-L pots and were placed in double rows in an experimental garden. Plants received either a high- or a low-nutrient treatment, which was achieved by mixing different concentrations of a slow-release fertilizer through a peaty, nutrient-poor potting soil (Osmocote Plus Mini [Scotts Europe BV, Waardenburg, The Netherlands]: 16N-8P-11K-2Mg including micronutrients, fertilizing activity three to four months; soil pH adjusted to 6.5). Pots were watered several times per day using an automated drip irrigation system (Netafim [Hatzerim, Israel] 4 L h<sup>-1</sup> pressure compensated drippers). The experiment followed a randomized complete block design, with eight blocks, two nutrient levels, and 150 genotypes (same as in field experiment). Each block contained one plant per nutrient level per genotype; only the parental accessions were included with two replicates. To provide seedlings with a standardized competitive environment (which was relevant in the context of analyzing fitness consequences of variation in relative growth rate, which is the focus of a separate manuscript), *H. spontaneum* seedlings were grown together with two *Avena sterilis* plants per pot. *Avena sterilis* is an annual wild oat species that coexists with *H. spontaneum* in Israel (Noy-Meir et al. 1989) and has a comparable growth form and phenology. Seeds from *A. sterilis* (Herbiseed, Twyford, England) were germinated and vernalized at 4°C for three weeks, grown for four days under greenhouse conditions, and subsequently stored at 5°C until outplanting. Transferring to experimental pots occurred simultaneously with *H. sponta-*

*neum* seedlings, at which time both species were of similar sizes.

Prior to germination, cleaned seeds were weighed individually. Under the experimental conditions, all *Hordeum* plants flowered and produced seeds. For each flowering plant, we determined the number of heads, the average number of seeds per head (in the first three heads), and mean seed weight (by collecting all matured seeds from one of these heads). Collected seeds were checked for the presence of a kernel to distinguish aborted from fertile seeds (in four blocks), and mean seed weight was determined for the fertile seeds (in all blocks). The number of heads was determined after plants had died by counting the number of tillers that exceeded the half-length of the tallest tiller. This excluded the smallest and last-developed tillers that generally produce few seeds and do not contribute much to the plant's total seed production, averaging 14.5% of all tillers per high-nutrient plant and 5.5% of all tillers per low-nutrient plant. Although the criterion for excluding small and late-developed tillers differed between the garden and the field experiments, the two methods appeared comparable: similar percentages of tillers were excluded in plants of similar size in the two experiments (e.g., compare plants grown at the ME site and under low-nutrient common garden conditions; see Results section).

#### Data Analysis

Generalized linear models were fit to assess the genotypic and environmental effects on the probability of germination and survival to reproduction (SAS, procedure GENMOD with a binomial error distribution and logit link function, Type III analysis). However, due to near 100% germination at the AQ site and near 100% survival at both sites, factorial models generally failed to converge. We therefore analyzed the joint effect of germination and survival as one viability fitness component (probability of survival for a planted seed), and we could fit only simplified models that excluded three-way interactions. Significance of model terms was determined via pseudo-*F*-tests based on the ratio of mean deviances of the model term and the appropriate error term as established in corresponding GLM models (cf. Schmid and Dolt 1994).

Analyses of variance (ANOVAs) were performed to assess the genotypic and environmental effects on the fecundity fitness scores of reproducing plants (SAS, procedure GLM, Type III EMS). In the field design, site, population of origin, harvest, and water treatment were fixed factors, while plot,  $F_3$  line, and accession were considered random factors. In the common-garden design, nutrient treatment and population of origin were fixed factors, while block,  $F_3$  line, and accession were random factors; initial seed weight was included as a covariate. Full-factorial models were fit with interactions with plot/block or harvest pooled in the error term. Prior to all analysis, data of the number of heads per plant and mean seed weight were log-transformed. The proportion of fertile seeds was near 100% in all four environments; these data were not subjected to ANOVA.

Broad-sense heritabilities of fecundity fitness components were estimated in the  $F_3$  lines using variance component analysis (SAS, procedure VARCOMP, REML estimation). Heritabilities were calculated within environments as the pro-

portion of variance explained by differences among  $F_3$  lines, after removing harvest and water variance in the field experiment. Significance of the heritability estimates was determined via the  $F_3$  line effect in corresponding GLM models.

Arithmetic line means for the fitness component traits were calculated within nutrient levels in the common-garden experiment. In the field experiment, due to unbalanced data, we calculated adjusted line means within each site from main-effect models accounting for harvest, water, and plot effects (LS means option in SAS, procedure GLM for fecundity traits and procedure GENMOD for viability fitness). Line means of total (integrated) fecundity fitness estimates were calculated as the product of the component trait line means, that is, seed number yield = heads  $\times$  seeds per head  $\times$  proportion fertile seeds and seed biomass yield = heads  $\times$  seeds per head  $\times$  proportion fertile seeds  $\times$  mean seed weight.

#### Quantitative Trait Locus Analysis

Taking the  $F_3$  line means as estimates for the trait value of their  $F_2$  parent, we performed QTL analysis using the  $F_2$  design in MapQTL 4.0 (van Ooijen and Maliepaard 1996). First, interval mapping analyses were carried out, followed by composite interval mapping (MQM) with markers near previously detected LOD peaks ( $> 2.0$ ) included in the analysis as cofactors. The MQM procedure was repeated with gradual addition of cofactors until the LOD profiles stabilized, and the final LOD scores were determined using restricted MQM. We used permutation tests to control for false positives caused by multiple tests during the genome scan. When looking for QTLs with opposite fitness effects in different environments, the penalty of making Type II errors (failure to detect QTLs that are really present) is relatively severe. As we aim to emphasize the general picture emerging from the complete set of QTLs rather than focusing on single loci, occasional Type I errors were considered less of a problem than repeated Type II errors. We therefore set a significance threshold that maintained a 10% mapwide error rate, corresponding to LOD-values between 2.8 and 3.8 for the different traits.

After QTLs had been identified, ANOVA models were used to formally test for QTL  $\times$  environment interactions. For this purpose,  $F_3$  lines were classified according to their genotype at the markers closest to the QTL, yielding two classes in the case of dominantly scored markers and three classes in the case of codominantly scored markers. ANOVA models were fit to explain variation in line mean scores by QTL marker, environment, and QTL marker  $\times$  environment effects.

We estimated mapwide heterozygosity (i.e., the proportion of heterozygous markers) and the mapwide proportion of ME alleles (arbitrarily chosen as the reference parent, equaling 1 - proportion AQ alleles) for each  $F_3$  line using available AFLP marker data, as described in the Appendix.

## RESULTS

### *Genotype $\times$ Environment Interactions for Fitness Traits* *Field experiment*

Due to a combination of edaphic and climatic factors (Table 1), the ME site was much more productive than the AQ

site, where plants typically produced one or two heads only (Fig. 1). In contrast, viability was higher at the AQ site, mainly due to higher germination (data not shown).

Analysis of the reciprocally transplanted accessions from the parental populations revealed significant effects of site and population of origin on all fitness components, except for the effect of population on viability (Table 2). Plants from the ME population produced more seeds per head than AQ plants, while the latter produced heavier seeds at both sites (Fig. 1A). Population of origin  $\times$  planting site interactions were significant for the fitness components number of heads per plant and mean seed weight, but not for viability and the number of seeds per head. ME plants produced more heads than AQ plants at the ME site, while head production hardly differed between the populations at the AQ site. The AQ plants' advantage of producing heavy seeds was greater at their native site than at the ME site. In neither environment, crossing reaction norms were observed for fecundity fitness component traits, but the multiplicative action of these components resulted in crossing reaction norms for total seed biomass yield per plant with native genotypes outperforming nonnative genotypes at both sites (ME site: 5.64 g for native ME plants and 4.88 g for nonnative AQ plants; AQ site: 0.41 g for native AQ plants and 0.23 g for nonnative ME plants; see Fig. 1B). These crossing reaction norms are based on the product of population mean values for the underlying component traits. Significance of the site  $\times$  origin interaction is suggested by ANOVA of total seed production at the individual-plant level, using the subset of plants with available scores for all component fitness traits (124 plants in analysis; site  $\times$  origin interaction for seed biomass yield:  $F_{1,7} = 17.0$ ,  $P < 0.01$ ; data log-transformed prior to analysis).

The 140  $F_3$  lines of the mapping population showed significant line, site, and site  $\times$  line effects for all fecundity fitness component traits and significant site and site  $\times$  line effects for viability (Table 3). Heritabilities of fitness traits were higher at ME than AQ. Transgressive segregation was observed for all component fitness traits, with the exception of mean seed weight (data not shown).

*Common-garden experiment*

Limiting the nutrient supply resulted in a reduction in population mean scores of all fecundity fitness traits (Fig. 1; Table 4). As in the field experiment, ME plants produced more seeds per head than AQ plants, while the latter produced heavier seeds under both nutrient conditions. The nutrient and population of origin effects on all component fitness traits were significant (Table 4). Origin  $\times$  nutrient interaction for the number of heads per plant seemed to parallel origin  $\times$  site interaction in the field experiment: the ME plants advantage of producing many heads was expressed mainly under high-nutrient conditions. No origin  $\times$  nutrient interaction was observed for the other fecundity fitness component traits. Reaction norms for total seed number and total seed biomass (calculated as the product of population means for the component fitness traits) did not cross. Analyzing seed number yield and seed biomass yield at the individual-plant level, using the subset of plants with available scores for all component fitness traits, indicated no significant nutrient  $\times$  origin

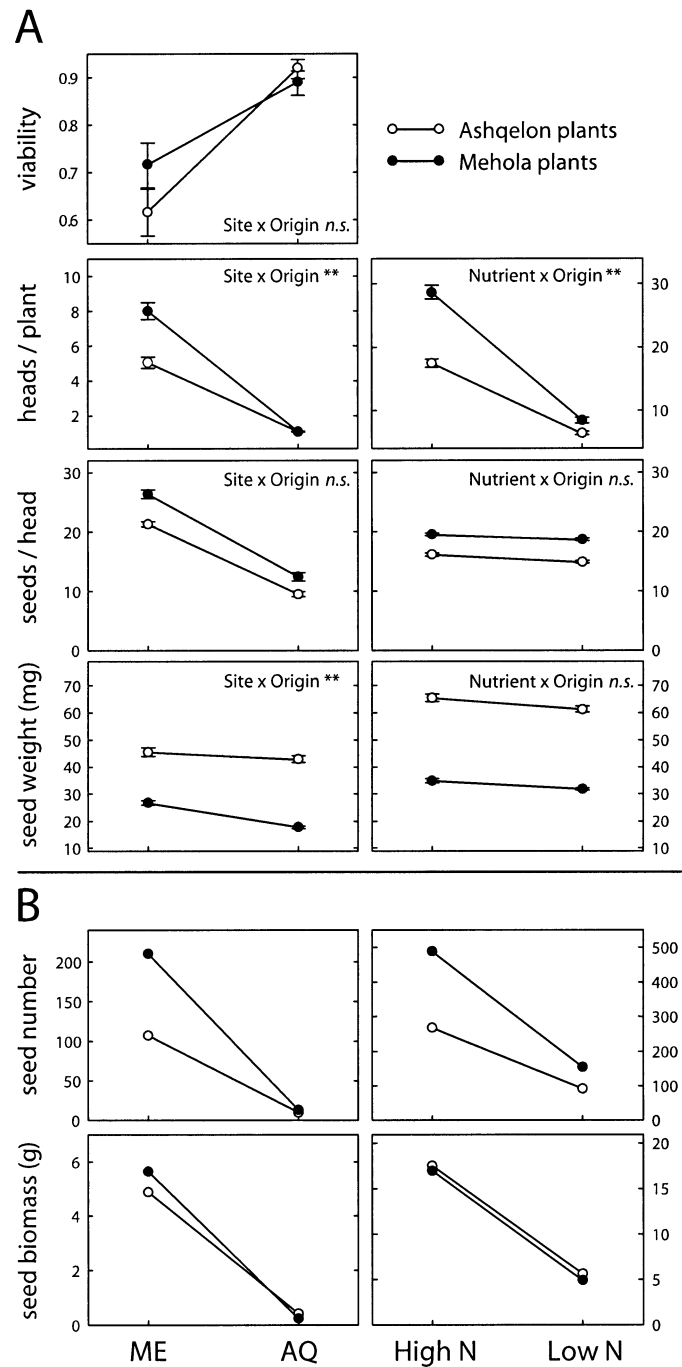


FIG. 1. Reaction norms for fitness component traits (A) and multiplicative seed yield estimates (B; calculated as the product of population means for the underlying component traits) of plants originating from the Mehola (ME) and Ashqelon (AQ) populations, reciprocally transplanted at the two field sites and grown under contrasting nutrient conditions in the common-garden experiment (population means  $\pm$  SE). Five accessions were grouped per population. Significance of environment  $\times$  origin interactions for the component fitness traits as in Tables 2 and 4.

TABLE 2. Field experiment: analysis of variation in performance of accessions from the Ashqelon and Mehola populations grown at both field sites. *F*-values indicate effects of site (S), population of origin (O), accession (A), initial water treatment (W), harvest, and plot on fitness traits. Denominator degrees of freedom are given in parentheses. The two accessions that were used as parents for the interpopulation cross were compared using a priori contrasts (boldface).

	df	Viability <i>n</i> = 570	Heads <i>n</i> = 421	Seeds per head <i>n</i> = 163	Mean seed weight <sup>1</sup> <i>n</i> = 220
Site	1	7.0 <sub>(11)</sub> *	59.0 <sub>(9)</sub> ***	161.6 <sub>(10)</sub> ***	15.6 <sub>(9)</sub> **
Origin	1	0.0 <sub>(8)</sub>	12.5 <sub>(8)</sub> **	13.2 <sub>(9)</sub> **	287.1 <sub>(8)</sub> ***
Accession(O)	8	0.2 <sub>(8)</sub>	1.4 <sub>(3)</sub>	1.3 <sub>(7)</sub>	0.6 <sub>(8)</sub>
<b>Parent</b>	<b>1</b>	<b>0.0<sub>(8)</sub></b>	<b>0.1<sub>(3)</sub></b>	<b>0.3<sub>(7)</sub></b>	<b>43.2<sub>(8)</sub>***</b>
S × O	1	1.1 <sub>(8)</sub>	12.5 <sub>(8)</sub> **	4.5 <sub>(10)</sub>	15.1 <sub>(8)</sub> **
S × A(O)	8	2.6 <sub>(531)</sub> ***	1.1 <sub>(8)</sub>	2.2 <sub>(8)</sub>	3.1 <sub>(8)</sub>
<b>S × parent</b>	<b>1</b>	<b>0.0<sub>(531)</sub></b>	<b>0.1<sub>(8)</sub></b>	<b>7.3<sub>(8)</sub>*</b>	<b>5.9<sub>(8)</sub>*</b>
Harvest <sup>2</sup>	1–3	—	2.3 <sub>(370)</sub>	15.2 <sub>(115)</sub> ***	0.3 <sub>(171)</sub>
Water	1	0.1 <sub>(8)</sub>	0.4 <sub>(8)</sub>	2.1 <sub>(8)</sub>	8.1 <sub>(7)</sub> *
Plot(S × W)	8	4.0 <sub>(531)</sub> ***	37.3 <sub>(370)</sub> ***	2.7 <sub>(115)</sub> **	1.6 <sub>(171)</sub>
O × W	1	0.1 <sub>(8)</sub>	0.0 <sub>(8)</sub>	0.1 <sub>(9)</sub>	0.1 <sub>(9)</sub>
A(O) × W	8	1.4 <sub>(531)</sub>	0.9 <sub>(8)</sub>	1.6 <sub>(7)</sub>	1.4 <sub>(8)</sub>
S × W	1	0.2 <sub>(8)</sub>	0.2 <sub>(9)</sub>	3.1 <sub>(7)</sub>	2.0 <sub>(6)</sub>
S × W × O	1	—	0.1 <sub>(8)</sub>	7.9 <sub>(10)</sub>	3.6 <sub>(9)</sub>
S × W × A(O)	8	—	2.2 <sub>(370)</sub> *	0.7 <sub>(115)</sub>	0.9 <sub>(171)</sub>

<sup>1</sup> Weighted analysis of variance using the number of weighed seeds as weight factor.

<sup>2</sup> Some traits were measured at two and some at four harvests.

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

effects (91 plants; nutrient × origin interaction for seed number yield:  $F_{1,8} = 0.4$ , ns; for seed biomass yield:  $F_{1,8} = 0.6$ , ns; data log-transformed prior to analysis).

In the 140 F<sub>3</sub> lines of the mapping population, significant line and nutrient effects were observed for all fecundity fitness components, with weakly significant line × nutrient interactions for seeds per head and mean seed weight (Table 5). Heritabilities were higher than in the field experiment (cf. Table 3), likely reflecting substantial spatial heterogeneity of the field sites. As in the field experiment, transgressive segregation was observed for all traits except mean seed weight (data not shown).

*Genomewide Effects of Heterozygosity and Proportion ME Alleles*

Independent effects of mapwide heterozygosity and the mapwide proportion of ME alleles were estimated by multiple regression of fecundity fitness line mean values on these two genotypic characteristics. This analysis may yield slightly

biased results, as linked markers are not independent. Nevertheless, fitting the same model in different environments permits interpretation of differences in results between environments. In the common-garden experiment, a positive fitness effect of the proportion heterozygous markers was observed at both nutrient levels and for both total seed output estimates (Fig. 2). In contrast, the heterozygosity level did not affect fitness under field conditions. In the field experiment, the proportion of ME alleles was positively related to seed number yield exclusively at the ME site. The proportion of ME alleles had opposite effects on seed biomass yield at the two sites, although the relationship was only significant at the AQ site (here, the negative regression coefficient indicated an advantage of a high proportion of AQ alleles; see Fig. 2). This is consistent with the crossing reaction norms for seed biomass yield observed at the population level. In the common-garden experiment, the proportion of ME alleles positively affected seed number yield but showed no association with seed biomass yield; this was observed at both

TABLE 3. Analysis of variation in performance of the 140 recombinant F<sub>3</sub> lines (L), grown at the Ashqelon (AQ) and Mehola (ME) field sites. See Table 2 for explanation. Heritabilities (*H*<sup>2</sup>) are from within-site analyses.

	df	Viability <i>n</i> = 8112	Heads <i>n</i> = 6202	Seeds per head <i>n</i> = 2593	Mean seed weight <i>n</i> = 2978
Site	1	13.1 <sub>(8)</sub> **	104.0 <sub>(8)</sub> ***	1095.5 <sub>(9)</sub> ***	56.9 <sub>(11)</sub> ***
Line	139	1.0 <sub>(72)</sub>	1.5 <sub>(84)</sub> *	2.0 <sub>(47)</sub> **	3.6 <sub>(57)</sub> ***
<b><i>H</i><sup>2</sup> AQ site</b>	—	—	<b>2.2%</b>	<b>5.0%</b>	<b>20.2%</b>
<b><i>H</i><sup>2</sup> ME site</b>	—	—	<b>4.7%</b>	<b>17.8%</b>	<b>22.6%</b>
S × L	139	1.5 <sub>(7683)</sub> ***	1.8 <sub>(139)</sub> ***	1.4 <sub>(131)</sub> *	1.7 <sub>(138)</sub> **
Harvest	1–3	—	7.2 <sub>(5631)</sub> ***	197.5 <sub>(2030)</sub> ***	0.4 <sub>(2410)</sub>
Water	1	0.1 <sub>(8)</sub>	0.7 <sub>(8)</sub>	6.7 <sub>(7)</sub> *	9.9 <sub>(8)</sub> *
Plot(S × W)	8	21.6 <sub>(7683)</sub> ***	200.6 <sub>(5631)</sub> ***	5.6 <sub>(2030)</sub> ***	6.6 <sub>(2410)</sub> ***
L × W	139	0.9 <sub>(7683)</sub>	1.0 <sub>(139)</sub>	0.7 <sub>(130)</sub>	0.7 <sub>(138)</sub>
S × W	1	0.2 <sub>(8)</sub>	0.3 <sub>(8)</sub>	4.2 <sub>(8)</sub>	0.0 <sub>(9)</sub>
L × S × W	139	—	1.2 <sub>(5631)</sub>	0.9 <sub>(2030)</sub>	1.2 <sub>(2410)</sub>

TABLE 4. Analysis of variation in performance of accessions from the Ashqelon and Mehola populations grown at different nutrient availabilities in the common-garden experiment. *F*-values indicate effects of nutrient treatment (N), population of origin (O), accession (A), and block on fitness traits. See also Table 2.

	df	Heads <i>n</i> = 185	Seeds per head <i>n</i> = 185	Mean seed weight <sup>1</sup> <i>n</i> = 184
Nutrient	1	1572.5 <sub>(10)</sub> ***	22.8 <sub>(8)</sub> **	5.5 <sub>(8)</sub> *
Origin	1	43.6 <sub>(33)</sub> ***	19.9 <sub>(10)</sub> **	163.1 <sub>(27)</sub> ***
Accession(O)	8	6.4 <sub>(9)</sub> **	14.5 <sub>(8)</sub> ***	1.6 <sub>(8)</sub>
<b>Parent</b>	<b>1</b>	<b>34.8<sub>(9)</sub>***</b>	<b>77.1<sub>(8)</sub>***</b>	<b>44.1<sub>(8)</sub>***</b>
N × O	1	11.4 <sub>(10)</sub> **	3.9 <sub>(8)</sub>	0.1 <sub>(8)</sub>
N × A(O)	8	0.5 <sub>(156)</sub>	1.7 <sub>(156)</sub>	2.7 <sub>(156)</sub> **
<b>N × parent</b>	<b>1</b>	<b>3.4<sub>(156)</sub></b>	<b>0.3<sub>(156)</sub></b>	<b>0.0<sub>(156)</sub></b>
Initial seed mass	1	10.3 <sub>(156)</sub> **	2.7 <sub>(156)</sub>	2.4 <sub>(156)</sub>
Block	8	2.4 <sub>(156)</sub> *	1.9 <sub>(156)</sub>	1.8 <sub>(156)</sub>

<sup>1</sup> Weighted analysis of variance using the number of weighed seeds as weight factor.

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

nutrient levels. When lifetime fitness scores were analyzed in the field experiment instead of fecundity fitness scores, calculated as the product of line mean scores for viability and seed number or seed biomass yield, similar results were obtained (data not shown).

#### Quantitative Trait Locus Analysis

##### Field experiment

For viability fitness only one QTL was found, at the AQ site (Fig. 3). For fecundity traits at the ME site, we detected three QTLs affecting the number of heads per plant (together explaining 34% of the observed variation in line mean scores); two QTLs for the number of seeds per head (21% of observed variation); three QTLs for mean seed weight (56% of observed variation); four QTLs for seed number yield (76% of observed variation); and four QTLs for seed biomass yield (43% of observed variation). At the AQ site fewer QTLs for fecundity traits were detected, explaining less of the observed variation in line means (number of heads: one QTL, 15%; mean seed weight: five QTLs, 62%; seed biomass yield: two QTLs, 28%), which is in agreement with low heritabilities for the traits observed at this site. Of the nine fitness-related QTLs detected at the AQ site, four seemed to overlap with QTLs at the ME site, while 12 additional QTLs were observed at the latter site only. Inspection of

additive effects at the QTLs for total yield estimates revealed that, at the AQ site, native AQ homozygotes outperformed ME homozygotes at both QTLs for seed biomass yield. At the ME site the same two QTLs for seed biomass yield were detected (with effects in the same direction, i.e., AQ homozygote superiority), but also two additional QTLs were found at which ME alleles increased biomass yield. All four QTLs for seed number yield at the ME site showed ME allele superiority. Observed *d/a* ratios suggested that overdominance (*d/a* > 1) as well as underdominance (*d/a* < -1) occurred frequently at the QTLs for total yield estimates. QTLs that were detected at both sites had effects in the same direction: no QTL was observed with opposite fitness effects in the two field environments.

##### Common-garden experiment

Under high-nutrient conditions, we detected three QTLs for the number of heads (accounting for 65% of observed line mean variation); two QTLs for the number of seeds per head (34% of observed variation); four QTLs for mean seed weight (48% of observed variation); three QTLs for seed number yield (40% of observed variation); and four QTLs for seed biomass yield (38% of observed variation; Fig. 3). Under low-nutrient conditions, fewer QTLs were found, explaining less of the total variation (9–45%; no QTL for seed biomass yield). The QTLs detected in the low-nutrient environment appeared to represent a subset of the QTLs detected under high-nutrient conditions: eight of nine QTLs from the low-nutrient environment showed overlapping LOD support intervals with QTLs in the high-nutrient environment. Irrespective of nutrient level, ME alleles increased the number of heads and the number of seeds per head, whereas AQ alleles increased mean seed weight at the respective QTL. At the QTLs for total seed number yield, ME homozygotes outperformed AQ homozygotes (except for one minor QTL on chromosome 3), while AQ homozygotes generally outperformed ME homozygotes at QTLs for total seed biomass yield (except for one QTL on chromosome 7). The majority of QTLs for total yield estimates showed *d/a* ratios greater than one, suggesting frequent overdominance. As in the field experiment, QTLs that were detected at both nutrient levels had effects of the same direction in the two environment; no QTLs were observed that showed significant opposite effects in the contrasting environments. QTLs for the number of seeds per head and for mean seed weight repeatedly co-located with alleles having opposite effects on the two traits

TABLE 5. Analysis of variation in performance of the 140 recombinant F<sub>3</sub> lines (L) grown at different nutrient availabilities in the common-garden experiment. See Table 4 for explanation. Heritabilities (*H*<sup>2</sup>) are from within-nutrient level analyses.

	df	Heads <i>n</i> = 2153	Seeds per head <i>n</i> = 2154	Mean seed weight <i>n</i> = 2149
Nutrient	1	13085.9 <sub>(142)</sub> ***	608.0 <sub>(142)</sub> ***	228.0 <sub>(144)</sub> ***
Line	139	2.1 <sub>(140)</sub> ***	17.5 <sub>(140)</sub> ***	6.1 <sub>(140)</sub> ***
<b><i>H</i><sup>2</sup> high-N</b>		<b>7.5%</b>	<b>59.6%</b>	<b>29.0%</b>
<b><i>H</i><sup>2</sup> low-N</b>		<b>5.1%</b>	<b>55.1%</b>	<b>40.8%</b>
N × L	139	1.1 <sub>(1865)</sub>	1.3 <sub>(1866)</sub> *	1.3 <sub>(1861)</sub> *
Initial seed mass	1	52.8 <sub>(1865)</sub> ***	28.5 <sub>(1866)</sub> ***	0.2 <sub>(1861)</sub>
Block	7	15.4 <sub>(1865)</sub> ***	7.1 <sub>(1866)</sub> ***	9.1 <sub>(1861)</sub> ***

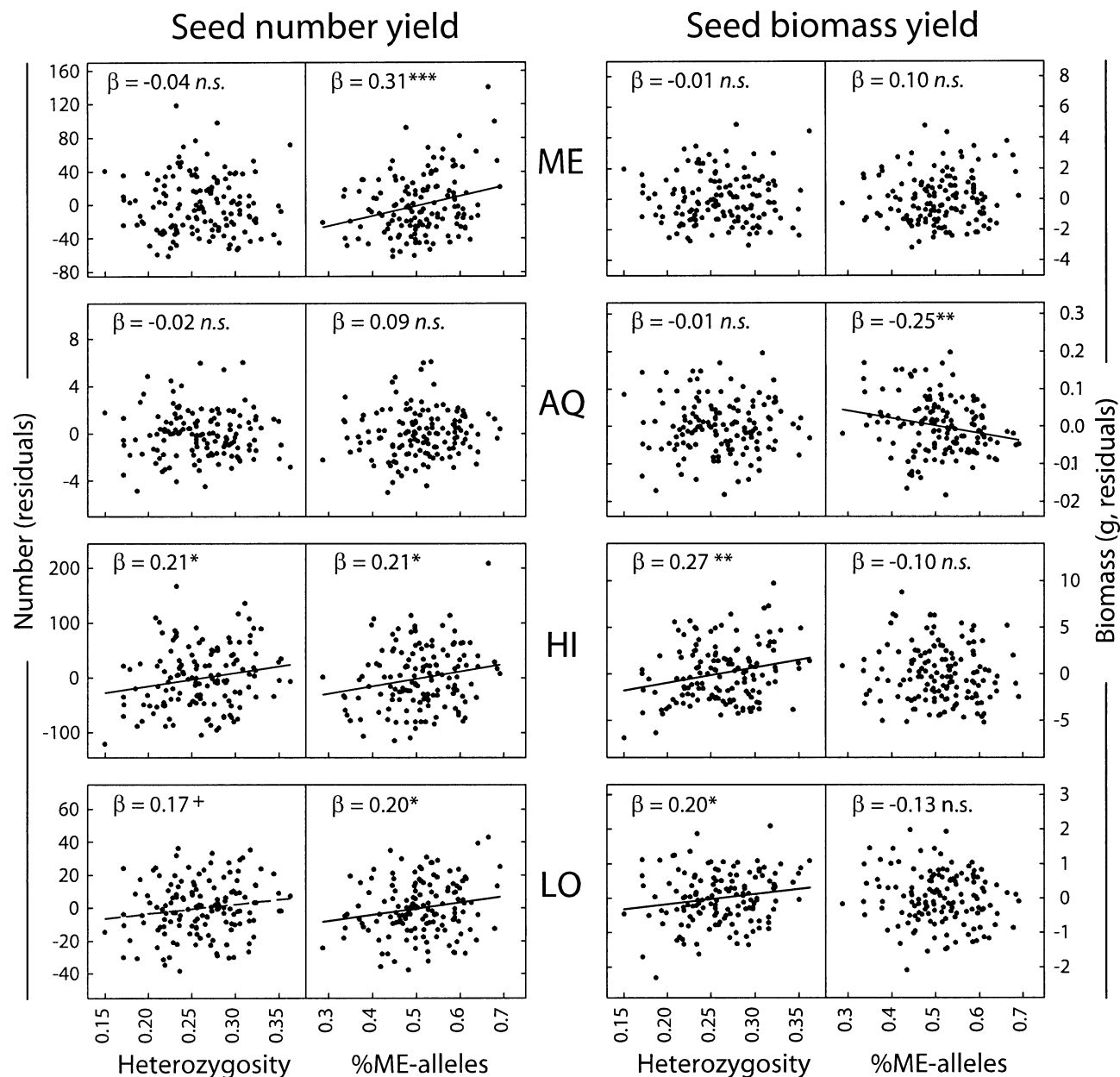


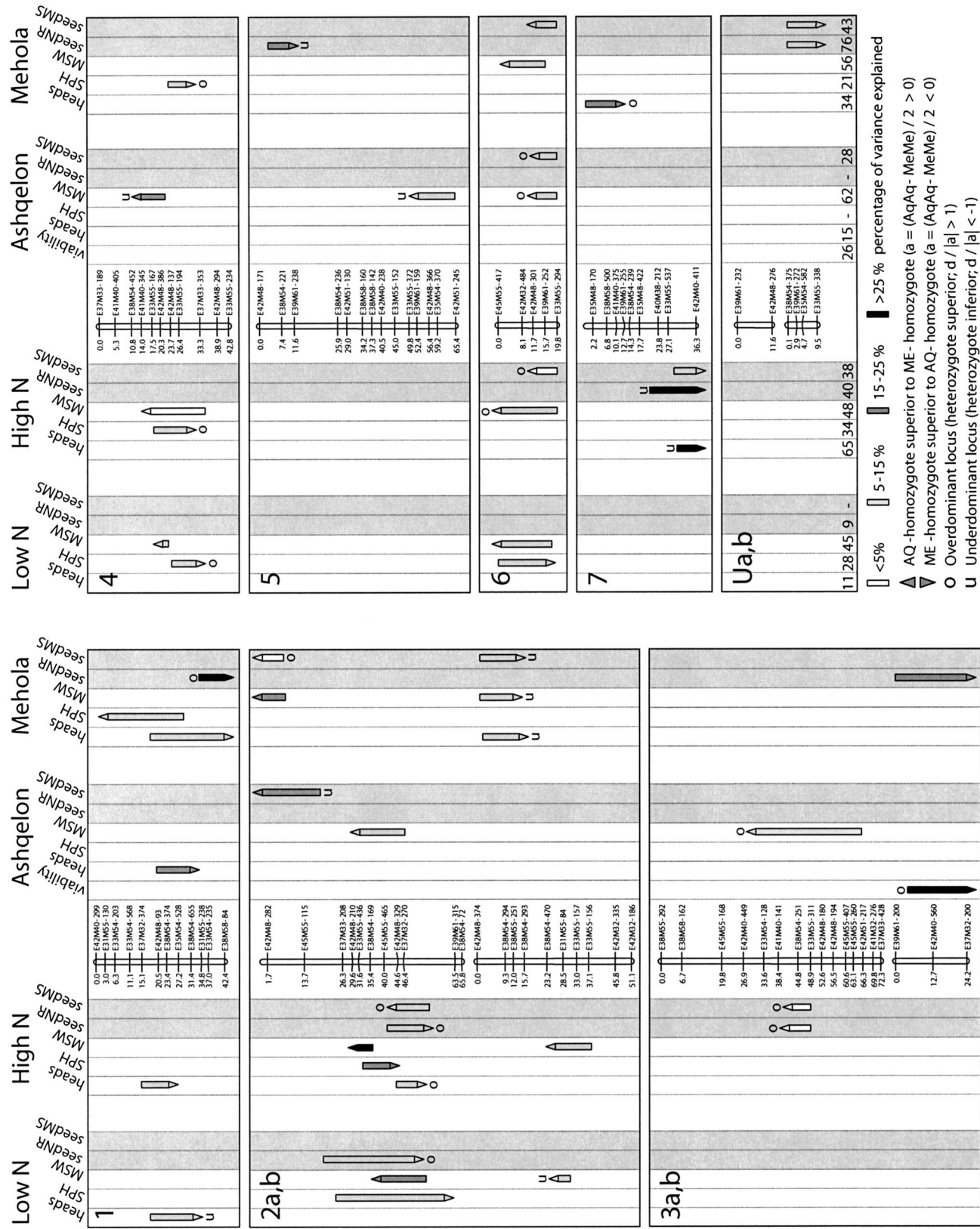
FIG. 2. Fitness effects of mapwide heterozygosity and the mapwide proportion of ME alleles at the Mehola (ME) and Ashqelon (AQ) field sites and under high (HI) and low (LO) nutrient conditions. Standardized partial regression coefficients ( $\beta$ ) are from within-environment multiple regression of yield on the two genetic predictor variables, based on 140  $F_3$  line means. Plots for individual predictor variables use residual yield scores obtained after regression on the other predictor (i.e., the relation between heterozygosity and yield is corrected for variation caused by %ME alleles, and vice versa). Significance of  $\beta$ -values: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; + $P < 0.10$ .

(see Fig. 3), strongly suggesting a pleiotropic basis for the observed trade-off between these traits (correlation of line mean values:  $r_{140} = -0.523$ ,  $P < 0.001$  at high nutrients;  $r_{140} = -0.512$ ,  $P < 0.001$  at low nutrients).

Very limited overlap was observed between experiments of QTLs governing total fecundity fitness. Many QTLs detected in the field were not found in the common-garden experiment, as indicated by the lack of overlap of the one-LOD support intervals (Fig. 3). Only one QTL for seed biomass yield on chromosome 6 was found in both experiments.

#### Quantitative Trait Locus $\times$ Environment Interactions

Because statistical power to detect QTLs in mapping populations of modest size is limited (Beavis 1994), a QTL may be detected in one environment but may be absent in another because it just did not reach the significance threshold. To explore the pattern of environmental specificity of QTLs (Fig. 3) in more detail, we compared the estimated additive effects across environments of loci that were declared QTL in at least one of the two environments (Fig. 4). If loci have the same direction and relative magnitude of effect in two con-



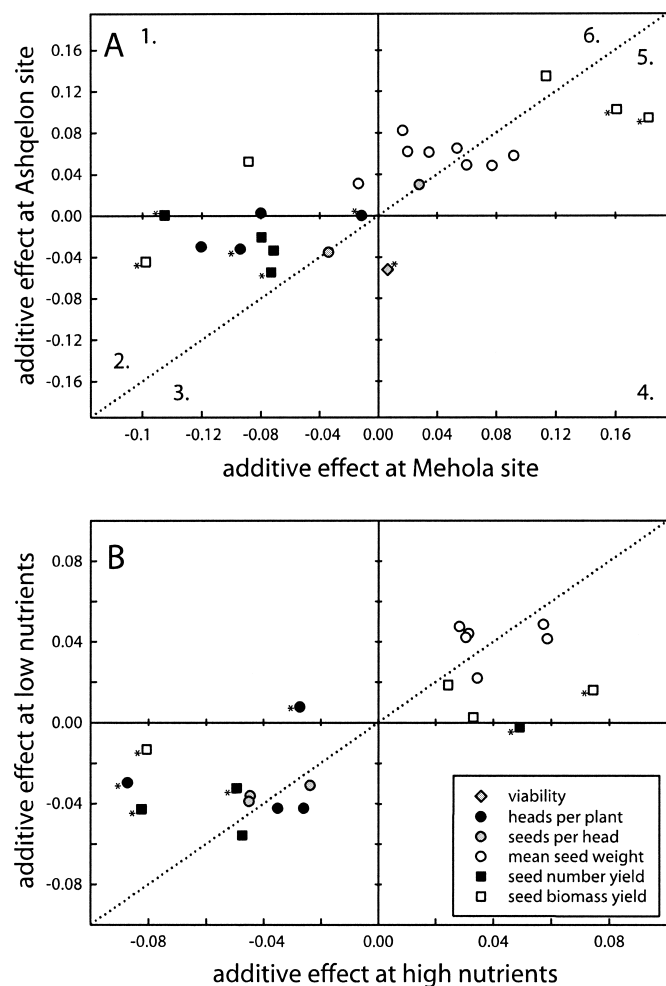


Fig. 4. Additive effects of QTLs that significantly affected fitness traits in at least one of two field sites (A) or in at least one of two nutrient environments (B). Additive effects were estimated using restricted MQM analysis in MapQTL, and were in each environment calculated as  $(AQAQ - MEME)/2$ . Hence, positive values indicate that the AQ allele has an increasing effect on the trait score, whereas negative values indicate that the ME allele has an increasing effect, irrespective of the test environment. To allow comparison between traits, additive effects are expressed relative to the midhomozygote value. Asterisks indicate loci that showed significant QTL  $\times$  environment interaction in GLM models. See text for interpretation of the six different plot areas.

trasting environments, that is, if they show no QTL  $\times$  environment interaction, they fall on the  $x = y$  diagonal in the plot. True environmental-specific QTLs will be located on the  $x$ - or  $y$ -axis. Individual loci are positioned in one of six possible plot areas, which are interpreted as follows for the

field experiment (see Fig. 4A): (1) ME alleles are favorable at the ME site, and AQ alleles are favorable at the AQ site; (2) ME alleles are favorable at both sites, but the effect on fitness is stronger at the ME site than the AQ site; (3) ME alleles are favorable at both sites, but the effect on fitness is stronger at the AQ site than the ME site; (4) ME alleles are favorable at the AQ site, and AQ alleles are favorable at the ME site; (5) AQ alleles are favorable at both sites, but the effect on fitness is stronger at the ME site than the AQ site; or (6) AQ alleles are favorable at both sites, but the effect on fitness is stronger at the AQ site than the ME site. Loci that deviate from the diagonal toward the upper left (plot areas 1, 2, and 6) can contribute to adaptive differentiation. Likewise, in the common-garden experiment (Fig. 4B), points in the upper-left quadrant of the graph indicate loci with ME allele superiority under nutrient-rich conditions and AQ allele superiority under nutrient-poor conditions, which might be expected under the assumption that ME genotypes are adapted to rich soil and AQ genotypes are adapted to poor soil. Figure 4A confirms that most QTLs of the field experiment have effects that differ in magnitude but not in the direction of their effect across the sites. The majority of the QTLs deviated to the upper-left side of the  $x = y$  diagonal (17 datapoints to upper left vs. seven points to lower right;  $\chi^2_1 = 4.1$ ,  $P < 0.05$ ), that is, alleles tended to confer a stronger fitness advantage at their native site. Some QTLs appeared to express a discernible effect at only one site. Two loci, for mean seed weight and for seed biomass yield, showed opposite effects that contribute to local adaptation, but their QTL  $\times$  environment interactions proved not significant in GLM models and they were picked up as QTLs in one environment only.

Also in the common-garden experiment, most QTLs differed in the magnitude but not in the direction of effect across the nutrient levels, with significant QTL  $\times$  environment interactions for some loci. Two loci showed QTL  $\times$  nutrient interactions for the number of heads produced per plant. At both loci, ME alleles increased fitness at high nutrient supply, while the locus effects were much smaller at limiting nutrient supply. Overall, points in the plot deviated equally to the upper-left side as to the lower-right side of the  $x = y$  diagonal (10 datapoints to upper left vs. 11 points to lower right;  $\chi^2_1 = 0.05$ , ns), thus not substantiating a general pattern of ME genotypic adaptation to nutrient-rich conditions and AQ adaptation to nutrient-poor conditions.

## DISCUSSION

### *Evidence for Adaptive Population Differentiation*

Based on inherent differences in reproductive traits (number of tillers, number and mean weight of seeds produced)

Fig. 3. Overview of all quantitative trait loci (QTLs) detected at the Mehola (ME) and Ashqelon (AQ) field sites and under high- and low-nutrient conditions of the common-garden experiment, for the following traits: viability; number of heads per plant (heads); number of seeds per head (NSH); mean seed weight (MSW); seed number yield (seedNR); seed biomass yield (seedMS). The position, estimated effect (i.e., the proportion of total line means variance explained by the QTL), the direction of the additive effect, and the occurrence of overdominance and underdominance (see Appendix) are given for each QTL. The total variance explained by all QTLs is given for each trait at the bottom of the lanes. The lengths of the QTL bars indicate one-LOD support intervals. Linkage groups were assigned to known *Hordeum* chromosomes 1–7 based on markers in common with previously published *H. vulgare* maps (H. Poorter, C. P. E. van Rijn, T. K. Vanhala, K. J. F. Verhoeven, Y. E. M. de Jong, P. Stam, and H. Lambers, unpubl. ms.).

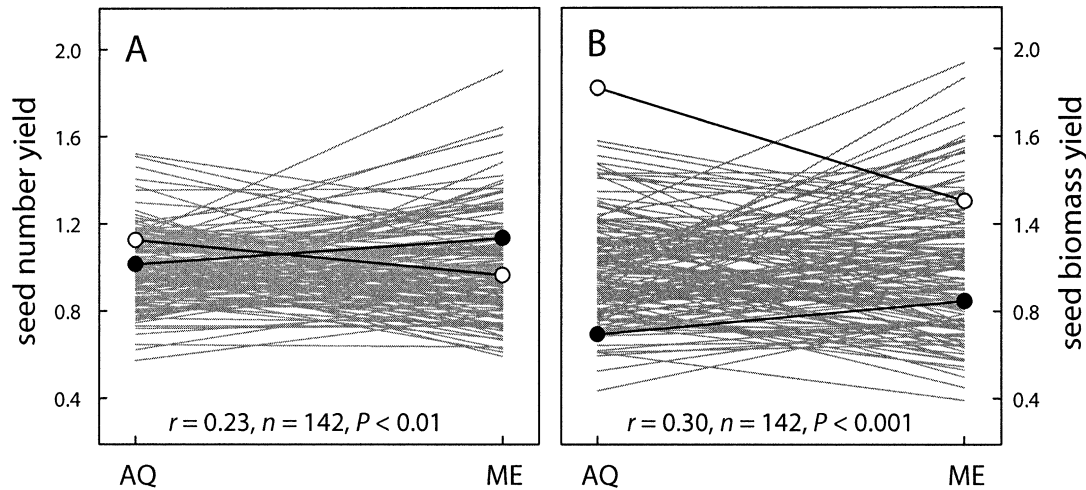


FIG. 5. Reaction norms for seed number yield (A) and seed biomass yield (B) in the field experiment for all  $F_3$  lines, the AQ parent (open circles), and the ME parent (filled circles). Yield scores are expressed as relative values by dividing each line score by the mean of all line scores in that environment to facilitate comparison of the two field sites (resultant scores have a mean value of one). Pearson correlations of line mean scores across the two sites are given.

the AQ and ME populations under study are consistent with different *H. spontaneum* ecotypes that are recognized in Israel (Snow and Brody 1984), which have been shown to be locally adapted (Volis et al. 2002a,b). In our study, reciprocal transplantation of accessions revealed genotype  $\times$  site interactions for several component fitness traits. The ME plants produced more heads than the AQ plants at their native ME site, but no difference in head production was observed at the AQ site. The AQ plants always produced heavier seeds than the ME plants, but this difference was expressed most clearly at the AQ site. For the multiplicative fitness estimates seed number yield and seed biomass yield, neither of the populations seemed to have been outperformed in its native habitat, whereas at least one population outperformed the introduced genotypes under native site conditions, a pattern consistent with the hypothesis of adaptive differentiation (Lortie and Aarssen 1996). The reaction norms for seed biomass yield crossed, albeit modestly, suggesting that native accessions outperformed introduced accessions at both field sites. The adaptive significance of genetic population differentiation was further indicated by a negative effect of dilution of the local gene pool, as measured by the proportion of native alleles at 96 marker loci dispersed over the entire linkage map, on seed number yield (ME site), and on seed biomass yield (AQ site). Although local adaptation and superiority of both populations at their native site can only be convincingly demonstrated in multiyear studies, our phenotypic and genetic results strongly suggest adaptive differentiation of the two populations.

#### Genetic Basis of Adaptive Differentiation

Many QTLs for fitness-related traits were detected under field conditions, but only few showed up at both the AQ site and the ME site, and none of these showed significant opposite effects in the two environments. Loci tended to differ in the magnitude but not in the direction of their effect across sites. Even though the same allele (at a specific fitness locus)

was generally beneficial at both field sites, the allele's fitness advantage was generally greater at its native site. It is the combined action of many such alleles that appears to underlie adaptive differentiation of the two populations. This main finding of our QTL analysis is in agreement with most previously reported QTL studies in crop species and in natural species tested in unnatural environments (see references in introduction). The results are consistent with the view that local adaptation in this species is based on multiple quantitative traits changing gradually across environments, without opposite selection on individual loci. We can speculate that such a genetic basis may typically underlie adaptive differentiation of populations along ecological gradients. Notably, genes with opposite fitness effects across environments have been detected in mapping populations derived from genotypes that had not adapted to different positions along an ecological gradient but to two alternative, discrete environments only (e.g., QTLs for pea aphid performance on two distinct hosts; Hawthorne and Via 2001). Adaptation along ecological gradients can also involve opposite-effect genes, but these might be observed most easily in populations from extreme opposite ends of the gradient (e.g., QTLs for maize performance in highland vs. lowland environments; Jiang et al. 1999).

The observation that loci with opposite fitness effects do not play a key role in adaptive differentiation in our model system predicts that recombinant genotypes can exist with near-optimal fitness in both field environments. Such genotypes would combine beneficial alleles at (nearly) all loci that affect fitness in either environment. Figure 5 shows the reaction norms of the recombinant  $F_3$  lines and the parental accessions for yield scores at the two field sites. Overall, fitness scores were positively correlated across the sites. For seed number yield, some recombinant lines appeared to outperform native parental accessions at both sites, and for both traits a large number of lines (36 for seed number yield and 42 for seed biomass yield) showed above-average fitness at

both sites. This is expected when loci of opposite fitness effect do not play a major role in population differentiation. In our sample of 140 recombinant lines, however, no single line was observed with optimal fitness at both sites. This may reflect the existence of opposite-effect loci that remained undetected in our QTL analysis, but it can also represent a sampling effect: many loci may affect fitness, even though not all are declared significant in QTL analysis, and an optimal genotype may not be present in our sample due to chance (e.g., if 10 loci affect fitness at AQ and/or ME, then only one of 1024 possible recombinants would be optimal).

The QTLs detected in this study generally explained less than 50% of the observed variance in line mean fitness scores. This may be chiefly attributed to the incomplete genome coverage of our chromosome map. As our map allowed detailed analysis of only 55% of the genome, almost half of the important QTLs segregating in the mapping population are expected to remain undetected in this study. Observed mean percentages of explained variance of 46% at the ME site and 45% at the high-nutrient level are therefore quite close to the expected maximum values and could suggest that in these environments most of the important QTLs on the visible part of the genome were indeed detected, but straightforward interpretation of such values is hampered by generally biased estimates of QTL effects (Beavis 1994). The incomplete map coverage restricts the conclusions that we can draw from the data, as we clearly cannot exclude the presence of opposite-effect QTLs in unseen parts of the genome.

A fitness advantage of increased heterozygosity in the  $F_3$  lines was observed in the common-garden experiment but not at either of the field sites. Heterozygosity advantage is expected upon crossing of inbred parental genotypes (Falconer and Mackay 1996) and has been reported in studies of similar design to ours (Fry et al. 1998; Jiang et al. 1999). The lack of heterozygosity advantage in our field experiment may reflect local adaptation: an inherently good performance of heterozygotes, as expressed under common-garden conditions, may be obscured under natural field conditions if homozygote genotypes confer a strong fitness advantage at their native site. Heterozygosity advantage under common-garden conditions appeared to be at least partly explained by overdominance at individual fitness loci. Although we did not perform significance tests for heterozygote superiority or inferiority, QTLs for total seed yield typically showed  $d/a$  ratios greater than one in the common-garden experiment, suggesting frequent overdominance. Heterozygote superiority at fitness QTLs has also been reported in other species (e.g., *Arabidopsis*: Mitchell-Olds 1995a; rice: Yu et al. 1997; Li et al. 2001).

#### *Population Differentiation Driven by Divergent Nutrient Availability?*

Population differentiation in *H. spontaneum* in Israel is believed to be at least partly driven by a north-to-south gradient in annual precipitation (Nevo et al. 1984; Snow and Brody 1984; Liviero et al. 2002). In our field study, adaptive differences in performance between the AQ and ME populations were observed that could not be easily related to dif-

ferences in precipitation: the two sites differed only modestly in total precipitation, and showed much higher productivity at the ME site that received the least rain but that showed highest soil nutrient availability. Comparing performance (at the population and at the QTL level) between the field experiment and the common-garden experiment, we explored the hypothesis that differentiation to divergent nutrient regimes has been a driving mechanism for adaptive differentiation between the field sites. In agreement with the hypothesis that the two populations have adapted to different nutrient availabilities, a population  $\times$  nutrient interaction was observed for the number of heads per plant that seemed to mimic a population  $\times$  site interaction observed in the field experiment. This was reflected at the genetic level in two QTLs that showed a significant QTL  $\times$  nutrient interaction for this trait, with ME alleles conferring a stronger fitness advantage under high-nutrient than low-nutrient conditions (Fig. 4). However, the general pattern of QTL effects at contrasting nutrient levels did not indicate an overall genotypic adaptation of ME and AQ plants to different nutrient levels. This suggests that population differentiation may be driven by selection to contrasting nutrient regimes only to a limited extent, and that other factors have contributed to adaptive differentiation as well.

In conclusion, our analysis of fitness effects of natural allelic variation in the native selection environments revealed the genetic basis of adaptive population differentiation in two populations of *H. spontaneum*. Although adaptive differentiation is often assumed to involve genetic trade-offs, they are not a necessary requirement for adaptive differentiation to evolve (Fry 1996; Kawecki 1997). In our study, loci with opposite fitness effect across environments played a very limited role in population differentiation; instead, adaptive genotype  $\times$  environment interaction was mainly caused by loci that differed in the magnitude and not the direction of their effect in the two native environments. These QTL results in natural populations are consistent with the view that differentiation may occur in the absence of genetic trade-offs. Additional studies, including populations from extreme ends of ecological gradients, could reveal whether this is in fact a common phenomenon.

#### ACKNOWLEDGMENTS

We thank T. Krugman, I. Fenton, A. Morgenstern, Y. Raines, and A. Hotzev for their invaluable logistical support in Israel. H. Turin, G. Disveld, S. Ivanovic, E. van Hoek, and T. Muller provided considerable technical assistance with the common-garden experiment. The study and the manuscript benefited from discussions with P. van Tienderen, S. Volis, P. Stam, A. van Noordwijk, and H.P. Koelewijn. The research was financially supported by the Earth and Life Sciences Research Council of the Netherlands Organization for Scientific Research (NWO-ALW project 805-33-244P). This paper is publication number 3238, NIOO-KNAW, Netherlands Institute of Ecology.

#### LITERATURE CITED

- Allard, R. W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. *J. Hered.* 79:225–238.

- Alonso-Blanco, C., S. E. D. El-Assal, G. Coupland, and M. Koornneef. 1998. Analysis of natural allelic variation at flowering time loci in the Landsberg *erecta* and Cape Verde islands ecotypes of *Arabidopsis thaliana*. *Genetics* 149:749–764.
- Beavis, W. D. 1994. The power and deceit of QTL experiments: lessons from comparative QTL studies. Pp. 250–266 in D. B. Wilkinson, ed. Forty-ninth annual corn and sorghum research conference. American Seed Trade Association, Chicago, IL.
- Bell, G., and X. Reboud. 1997. Experimental evolution in *Chlamydomonas* II. Genetic variation in strongly contrasted environments. *Heredity* 78:498–506.
- Bennington, C. C., and J. B. McGraw. 1995. Natural selection and phenotypic differentiation in *Impatiens pallida*. *Ecol. Monogr.* 65:303–323.
- Brown, A. H. D., D. Zohary, and E. Nevo. 1978. Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. *Heredity* 41:49–62.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237–268.
- Courtois, B., G. McLaren, P. K. Sinha, K. Prasad, R. Yadav, and L. Shen. 2000. Mapping QTLs associated with drought avoidance in upland rice. *Mol. Breed.* 6:55–66.
- Danin, A. 1988. Flora and vegetation of Israel and adjacent areas. Pp. 129–157 in Y. Yom-Tov and E. Tchernov, eds. The zoogeography of Israel. Dr W. Junk Publishers, Dordrecht, The Netherlands.
- Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman Scientific and Technical, Harlow, U.K.
- Fry, J. D. 1996. The evolution of host specialization: Are trade-offs overrated? *Am. Nat.* 148:S84–S107.
- Fry, J. D., S. V. Nuzhdin, E. G. Pasyukova, and T. F. C. McKay. 1998. QTL mapping of genotype-environment interaction for fitness in *Drosophila melanogaster*. *Genet. Res.* 71:133–141.
- Galen, C., J. S. Shore, and H. Deyoe. 1991. Ecotypic divergence in alpine *Polemonium viscosum*: genetic structure, quantitative variation, and local adaptation. *Evolution* 45:1218–1228.
- Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics* 121:129–138.
- Harlan, J. R., and D. Zohary. 1966. Distribution of wild wheats and barley. *Science* 153:1074–1080.
- Hawthorne, D. J., and S. Via. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* 412:904–907.
- Hayes, P. M., B. H. Liu, S. J. Knapp, F. Chen, B. Jones, T. Blake, J. Franckowiak, D. Rasmusson, M. Sorrells, S. E. Ullrich, D. Wesenberg, and A. Kleinhofs. 1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. *Theor. Appl. Genet.* 87:392–401.
- Hedrick, P. W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.* 17:535–566.
- Huang, Q., A. Beharav, Y. Li, V. Kirzhner, and E. Nevo. 2002. Mosaic microecological differential stress causes adaptive microsatellite divergence in wild barley, *Hordeum spontaneum*, at Neve Yaar, Israel. *Genome* 45:1216–1229.
- Jiang, C., G. O. Edmeades, I. Armstead, H. R. Lafitte, M. D. Hayward, and D. Hoisington. 1999. Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers. *Theor. Appl. Genet.* 99:1106–1119.
- Johnson, W. C., and P. Gepts. 2002. The role of epistasis in controlling seed yield and other agronomic traits in an Andean × Mesoamerican cross of common bean (*Phaseolus vulgaris* L.). *Euphytica* 125:69–79.
- Jordan, N. 1992. Path analysis of local adaptation in two ecotypes of the annual plant *Diodia teres* Walt. (Rubiaceae). *Am. Nat.* 140:149–165.
- Joshi, A., and J. N. Thompson. 1995. Trade-offs and the evolution of host specialization. *Evol. Ecol.* 9:82–92.
- Kawecki, T. J. 1997. Sympatric speciation via habitat specialization driven by deleterious mutations. *Evolution* 51:1751–1763.
- Kindell, C. E., A. A. Winn, and T. E. Miller. 1996. The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. *J. Ecol.* 84:745–754.
- Lander, E. S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.
- Lavie, B., V. Stow, T. Krugman, A. Beiles, and E. Nevo. 1994. Fitness in wild barley from two opposing slopes of a Mediterranean microsite at Mount Carmel, Israel. *Barley Genet. Newsl.* 23:12–14.
- Leips, J., and T. F. C. Mackay. 2000. Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. *Genetics* 155:1773–1788.
- Li, Z. K., L. J. Luo, H. W. Mei, D. L. Wang, Q. Y. Shu, R. Tabien, D. B. Zhong, C. S. Ying, J. W. Stansel, G. S. Khush, and A. H. Paterson. 2001. Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* 158:1737–1753.
- Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27:237–277.
- Liviero, L., E. Maestri, M. Gulli, E. Nevo, and N. Marmioli. 2002. Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. *Genet. Resour. Crop Ev.* 49:133–144.
- Lortie, C. J., and L. W. Aarssen. 1996. The specialization hypothesis for phenotypic plasticity in plants. *Int. J. Plant Sci.* 157:484–487.
- Lu, C., L. Shen, Z. Tan, Y. Xu, P. He, Y. Chen, and L. Zhu. 1996. Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled haploid population. *Theor. Appl. Genet.* 93:1211–1217.
- Mitchell-Olds, T. 1995a. Interval mapping of viability loci causing heterosis in *Arabidopsis*. *Genetics* 140:1105–1109.
- . 1995b. The molecular basis of quantitative genetic variation in natural populations. *Trends Ecol. Evol.* 10:324–328.
- Montalvo, A. M., and N. C. Ellstrand. 2001. Nonlocal transplantation and outbreeding depression in the shrub *Lotus scoparius* (Fabaceae). *Am. J. Bot.* 88:258–269.
- Nagy, E. S., and K. J. Rice. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* 51:1079–1089.
- Nevo, E. 1992. Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. Pp. 19–43 in P. R. Shewry, ed. *Barley: genetics, biochemistry, molecular biology and biotechnology*. C.A.B. International, Wallingford, U.K.
- Nevo, E., A. Beiles, Y. Gutterman, N. Storch, and D. Kaplan. 1984. Genetic resources of wild cereals in Israel and vicinity. II. Phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. *Euphytica* 33:737–756.
- Nevo, E., A. Beiles, D. Kaplan, E. M. Golenberg, L. Olsvig-Whittaker, and Z. Naveh. 1986a. Natural selection of allozyme polymorphisms: a microsite test revealing ecological genetic differentiation in wild barley. *Evolution* 40:13–20.
- Nevo, E., A. Beiles, and D. Zohary. 1986b. Genetic resources of wild barley in the Near East: structure, evolution and application in breeding. *Biol. J. Linn. Soc.* 27:355–380.
- Noy-Meir, I., M. Gutman, and Y. Kaplan. 1989. Responses of Mediterranean grassland plants to grazing and protection. *J. Ecol.* 77:290–310.
- Saranga, Y., M. Menz, C. X. Jiang, R. J. Wright, D. Yakir, and A. H. Paterson. 2001. Genomic dissection of genotype × environment interactions conferring adaptation of cotton to arid conditions. *Genome Res.* 11:1988–1995.
- Sari-Gorla, M., T. Calinski, Z. Kaczmarek, and P. Krajewski. 1997. Detection of QTL × environment interaction in maize by a least squares interval mapping method. *Heredity* 78:146–157.
- Schmid, B., and C. Dolt. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution* 48:1525–1549.
- Snow, L., and T. Brody. 1984. Genetic variation of *Hordeum spontaneum* in Israel: eco-geographic races, detected by trait measurements. *Plant Syst. Evol.* 145:15–28.

Teulat, B., O. Merah, I. Souyris, and D. This. 2001. QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. *Theor. Appl. Genet.* 103:774–787.

van Ooijen, J. W., and C. Maliepaard. 1996. MapQTL version 4.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen, The Netherlands.

van Tienderen, P. H., and J. van der Toorn. 1991. Genetic differentiation between populations of *Plantago lanceolata*. I. local adaptation in three contrasting habitats. *J. Ecol.* 79:27–42.

Volis, S., S. Mendlinger, and D. Ward. 2002a. Adaptive traits of wild barley plants from Mediterranean and desert origin. *Oecologia* 133:131–138.

Volis, S. 2002b. Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. *Biol. J. Linn. Soc.* 77:479–490.

Waser, N. M., and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evolution* 43: 1097–1109.

Wu, R. L. 1998. The detection of plasticity genes in heterogeneous environments. *Evolution* 52:967–977.

Xing, Y. Z., Y. F. Tan, J. P. Hua, X. L. Sun, C. G. Xu, and Q. Zhang. 2002. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor. Appl. Genet.* 105:248–257.

Yu, S. B., J. X. Li, Y. F. Tan, Y. J. Gao, X. H. Li, Q. F. Zhang, and M. A. S. Maroof. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc. Natl. Acad. Sci. USA* 94:9226–9231.

Zohary, D. 1969. The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World. Pp. 47–66 in P. J. Ucko and G. W. Dimbleby, eds. *The domestication and exploitation of plants and animals*. Duckworth, London.

Corresponding Editor: L. Galloway

APPENDIX

Linking F<sub>3</sub> phenotypic data to F<sub>2</sub> genotypes necessitated adjustment of QTL dominance effects as estimated by MapQTL and modification of the mapwide proportions of heterozygous markers and ME alleles. In the following, A and B denote the Ashqelon (AQ) allele and the Mehola (ME) allele at a marker locus, respectively. The dominance effect is calculated as:

$$d = \bar{x}_{AB} - (\bar{x}_{AA} + \bar{x}_{BB})/2. \tag{A1}$$

Homozygote genotypes in the F<sub>2</sub> are also homozygote in the F<sub>3</sub> after selfing, so MapQTL’s estimates (van Ooijen and Maliepaard 1996) of  $\bar{x}_{AA}$  and  $\bar{x}_{BB}$  are correct. However,  $\bar{x}_{AB}$  is estimated incorrectly as heterozygote F<sub>2</sub> genotypes segregate into ¼ AA, ½ AB, and ¼ BB plants. The expected phenotype of a heterozygote F<sub>2</sub> plant is:

$$\hat{x} = \mu_0 + \mu_A + \mu_B + \delta_{AB}, \tag{A2}$$

TABLE A1. Marker genotypic probabilities in F<sub>3</sub> lines given observed marker genotypes in F<sub>2</sub> plants.

F <sub>2</sub> marker (observed)	Mapwide count (n)	F <sub>2</sub> genotypic probability			F <sub>3</sub> genotypic probability		
		AA	AB	BB	AA	AB	BB
AA	a	1/1	—	—	1/1	—	—
BB	b	—	—	1/1	—	—	1/1
B ·	c	—	2/3	1/3	1/6	1/3	1/2
A ·	d	1/3	2/3	—	1/2	1/3	1/6
AB	h	—	1/1	—	1/4	1/2	1/4

where  $\mu_0$  is the overall mean,  $\mu_A$  is the additive effect of allele A,  $\mu_B$  is the additive effect of allele B, and  $\delta_{AB}$  is the dominance effect.

Due to segregation, the expected phenotype of F<sub>3</sub> progeny of this plant becomes:

$$\hat{x} = \frac{1}{4}(\mu_0 + 2\mu_A) + \frac{1}{2}(\mu_0 + \mu_A + \mu_B + \delta_{AB}) + \frac{1}{4}(\mu_0 + 2\mu_B) = \mu_0 + \mu_A + \mu_B + \frac{1}{2}\delta_{AB}. \tag{A3}$$

Substitution in (A1) gives  $d = \frac{1}{2}\delta_{AB}$ . MapQTL’s estimates of  $d$  were therefore multiplied by two to obtain the true  $\delta_{AB}$  dominance effect.

Using 96 markers of the core map, the mapwide proportion of heterozygotes is determined as:

$$\text{heterozygosity} = \frac{\text{no. AB}}{\text{no. AA} + \text{no. AB} + \text{no. BB}} \tag{A4}$$

and the mapwide proportion of ME alleles is determined as:

$$\begin{aligned} &\text{proportion ME alleles} \\ &= \frac{2(\text{no. BB}) + 1(\text{no. AB})}{2(\text{no. AA} + \text{no. AB} + \text{no. BB})}. \end{aligned} \tag{A5}$$

Given normal segregation, Table A1 shows the genotypic probabilities in the F<sub>3</sub> for each possible F<sub>2</sub> marker genotype, which differs for codominantly (*a, b, h*) and dominantly (*c, d*) scored markers. Accordingly, the mapwide heterozygosity and proportion of ME alleles for each F<sub>3</sub> line were calculated as:

$$\text{heterozygosity} = \frac{\frac{1}{3}c + \frac{1}{3}d + \frac{1}{2}h}{a + b + c + d + h} \tag{A6}$$

proportion ME alleles

$$= \frac{2\left(b + \frac{1}{2}c + \frac{1}{6}d + \frac{1}{4}h\right) + 1\left(\frac{1}{3}c + \frac{1}{3}d + \frac{1}{2}h\right)}{2(a + b + c + d + h)}. \tag{A7}$$

In the F<sub>3</sub> lines, estimated heterozygosity ranged from 15% to 36%; the estimated proportion of ME alleles ranged from 28% to 69%.