Distinguishing adaptive from nonadaptive genetic differentiation: comparison of Q_{ST} and F_{ST} at two spatial scales

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Genetic differentiation in 20 hierarchically sampled populations of wild barley was analyzed with quantitative traits, allozymes and Random Amplified Polymorphic DNAs (RAPDs), and compared for three marker types at two hierarchical levels. Regional subdivision for both molecular markers was much lower than for quantitative traits. For both allozymes and RAPDs, most loci exhibited minor or no regional differentiation, and the relatively high overall estimates of the latter were due to several loci with exceptionally high regional differentiation. The allozyme- and RAPD-specific patterns of differentiation were concordant in general with one another, but not with quantitative trait differentiation. Divergent selection on quantitative traits inferred from very high regional Q_{ST} was in full agreement with our previous results obtained from a test of local adaptation and multilevel selection analysis. In contrast, most variation in allozyme and RAPD variation was neutral, although several allozyme loci and RAPD markers were exceptional in their levels of regional differentiation. However, it is not possible to answer the question whether these exceptional loci are directly involved in the response to selection pressure or merely linked to the selected loci. The fact that Q_{ST} and F_{ST} did not differ at the population scale, that is, within regions, but differed at the regional scale, for which local adaptation has been previously shown, implies that comparison of the level of subdivision in quantitative traits, as compared with molecular markers, is indicative of adaptive population differentiation only when sampling is carried out at the appropriate scale.
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Introduction

Evolutionary forces determining population subdivision and the extent of genetic variation include those influencing all loci equally (migration, genetic drift and mating system) and those affecting loci differentially (natural selection and mode of inheritance). Cavalli-Sforza (1966) introduced the idea that heterogeneity of population differentiation (F_{ST}) values across loci reflects the involvement of different loci in an adaptive process. Subsequently, Lewontin and Krakauer (1973) further developed this idea into a formal statistical test. Until recently, allozyme loci encoding functional enzymes were the only molecular markers available to examine this idea, and, despite the continuing debate about possible direct selection effects on allozymes (Lewontin, 1991), allozymes have been assumed to be selectively neutral in most studies. The quantitative traits that represent additive or interactive effects of many contributing loci are more directly related to fitness than molecular markers; that is, while many may be neutral, some quantitative traits are adaptive. Selectively neutral quantitative traits are predicted to behave similarly to traits determined by a single locus (Rogers and Harpending, 1983). This led to the idea of testing selective divergence in quantitative traits by comparison of their population differentiation (Q_{ST}) with the population differentiation obtained from neutral molecular markers (F_{ST}) (Spitze, 1993). Three outcomes, each having a unique interpretation, are possible (Merila and Crnokrak, 2001): (i) $Q_{ST} > F_{ST}$: this is commonly interpreted as evidence of divergent selection and adaptation to local environments (Podolsky and Holtsford, 1995; Bonnin *et al*, 1996; Latta and Mitton, 1997; Jaramillo-Correa *et al*, 2001; Steinger *et al*, 2002; Storz, 2002; Chan and Arcese, 2003; Luttikhuizen *et al*, 2003); (ii) Q_{ST} and F_{ST} do not differ: genetic drift alone is sufficient to explain the pattern of differentiation (Yang *et al*, 1996), although effects of natural selection and drift in certain cases can be indistinguishable in certain cases (Sokal and Wartenburg, 1983); and (iii) $Q_{ST} < F_{ST}$: convergent selection favoring the same phenotype in different environments is assumed (Kuittinen *et al*, 1997; Petit *et al*, 2001; Edmands and Harrison, 2003). However, interpretation of any discrepancy between Q_{ST} and F_{ST} as evidence of selection on a particular quantitative trait may not always hold. As variation for quantitative traits

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is introduced by mutation at a higher rate than for molecular markers (Kimura, 1983; Lynch, 1988), the extent of variation for quantitative traits usually exceeds that for molecular markers (Morgan et al, 2001). The population divergence due to stochastic processes such as drift and founder effects may be detectable with quantitative traits but not with molecular markers when the latter possess a low level of polymorphism or when the genes involved in the expression of quantitative traits have significant epistatic effects (Lynch, 1994). One way to clarify the issue is to conduct a test of local adaptation. Unfortunately, no rigorous test of local adaptation has been conducted in a comparative study of Q_{ST} and F_{ST}.

Different classes of molecular markers may also differ in the level of population differentiation they reveal because of differences in mutation rate, neutrality or linkage disequilibrium with other loci that are subject to selection. In several studies, lower G_{ST} (between population diversity) values were found for allozymes than Random Amplified Polymorphic DNAs (RAPDs) (Ayres and Ryan, 1997; Latta and Mitton, 1997; Comes and Abbott, 2000), although other studies detected no difference (Isabel et al, 1995; Szmidt et al, 1996; Le Corre et al, 1997; Aagaard et al, 1998) or lower population differentiation in RAPDs has occasionally been detected (Allendorf and Seeb, 2000; Bartish et al, 2000b). As with quantitative vs DNA/allozyme markers, discordant patterns of divergence in several molecular markers and loci are commonly interpreted as the differential effect of natural selection on those markers and loci (Karl and Avise, 1992; McDonald, 1994; Latta and Mitton, 1997), despite no direct evidence of the latter.

Not only is the degree of population differentiation measured by F_{ST} or Q_{ST} important for understanding population genetics but also the pattern of population differentiation and especially the concordance (or discordance) in patterns of differentiation revealed by different markers. In a recent study where desert and Mediterranean plants of Hordeum spontaneum were found to be adapted to their locations and differentiated in phenotypic traits (Volis et al, 2002b), no concordance between quantitative and molecular (allozyme) pairwise genetic distances was detected (Volis et al, 2002a). Several studies in which patterns of quantitative trait variation were strongly associated with climate or distinct habitats also demonstrated a lack of correspondence between molecular and quantitative trait variation patterns (Karhu et al, 1996; Knapp and Rice, 1998; McKay et al, 2001; Steinger et al, 2002). In other studies where genetic subdivisions at quantitative and molecular markers were compared in their relation to geographic subdivision but without tests of local adaptation/ association with environmental parameters, or were compared without any reference to spatial location/ environmental differences, the outcomes varied from a close match (Lagercrantz and Ryman, 1990; Kuittinen et al, 1997; Morgan et al, 2001) to complete disagreement (Podolsky and Holtsford, 1995; Black-Samuelsson et al, 1997; Heaton et al, 1999; Storz, 2002). When only allozyme and RAPD population differentiation patterns based on genetic distances were compared, the correspondence varied from low (Mamuris et al, 1999; Sun and Wong, 2001; Vandewoestijne and Baguette, 2002) to very high (Peakall et al, 1995; Lifante and Aguinagalde, 1996; Jenczewski et al, 1999).

There is a need for a robust approach to verify the common interpretations of observed patterns of differentiation listed above, viz. $Q_{ST} > F_{ST}$ as evidence of divergent selection and $Q_{ST} < F_{ST}$ as evidence of convergent selection (Bossart and Pashley Prowell, 1998; Merila and Crnokrak, 2001). To date, a limited number of studies have undertaken the development of such an approach (Ross, 1997; Ross et al, 1997, 1999; McKay et al, 2001; Volis et al, 2002a, b).

Self-fertilization is a life history character reducing effective population size and frequency of recombination and limiting efficient migration to seed dispersal only. Thus, self-fertilization decreases within-population diversity and greatly increases the potential for population genetic structuring as compared with outcrossing (Charlesworth, 2003). Local selection will exacerbate the above effects, leading to large allele frequency differences, and high F_{ST} values in a large proportion of marker loci (Charlesworth et al, 1997). As pointed out by Charlesworth (2003) this implies that it is particularly difficult to identify loci subject to selection in inbreeders. Therefore, a comparison of F_{ST} and Q_{ST} in a selfing species is a more conservative test for neutral selectivity than in an outcrossing species because only strong local selection will cause $Q_{ST} > F_{ST}$ in a selfer, while drift alone or moderate selection will result in approximately equal and high values of Q_{ST} and F_{ST}. On the other hand, the level of population subdivision may differ at different geographical (environmental) scales, and sampling may or may not be relevant to the actual scale at which adaptive differentiation occurs. Therefore, a selfing species sampled at several hierarchical levels may provide insights into how much (if any) local selection contributes to population subdivision in Q_{ST} and F_{ST} at the level relevant to local adaptation.

The present study extends our previous studies of life history and population genetic differentiation in wild barley (H. spontaneum Koch), sampled using hierarchical design in four different environments (regions) representing distinct climatic zones and vegetation types. The 20 sampled populations (five populations per region) were analyzed for allozyme and RAPD variation (Volis et al, 2001, 2003) and quantitative traits (this paper). As a multiyear reciprocal introduction of seeds and seedlings conducted at one (pivot) location per region revealed local adaptation (Volis et al, 2002d), interpretation of the observed quantitative, allozyme and RAPD differentiation can be performed on the basis of a known effect of region-specific natural selection. We have the following objectives: (1) analysis of the extent and structure of quantitative trait variation; (2) a comparison of marker-specific (quantitative, allozyme and RAPD) estimates of regional genetic diversity, level and pattern of differentiation at two hierarchical levels; (3) interpretation of intermarker variation as due to selective or stochastic processes. Wild barley is a predominant selfing species that exhibits strong population differentiation in phenotypic traits (Snow and Brody, 1984), allozymes (Brown et al, 1978a), RAPDs (Baum et al, 1997), SSRs (Turpeinen et al, 2001) and AFLP markers (Pakniyat et al, 1997). High population subdivision due to selfing and genetic drift that is detectable with neutral markers can potentially mask population differentiation due to diversifying selection, making the Q_{ST} vs F_{ST} comparison inconclusive. Our major prediction was that overall estimates of

regional subdivision (a spatial scale where adaptation was found to occur) will be distinctly higher for quantitative than for either allozymes or RAPDs markers, while population differentiation within regions in Q_{ST} and F_{ST} will not differ.

Materials and methods

Study populations

H. spontaneum Koch is an annual, diploid grass with an estimated self-fertilization rate of 98% (Brown *et al*, 1978b). In Israel, despite its mainly Mediterranean and Irano-Turanian distribution in steppe-like formations, wild barley penetrates into desert (<200 mm annual rainfall) and mountain (up to 1600 m elevation) environments where it has stable populations. The plant material used in this study consisted of 20 populations sampled in Israel in 1996, employing a nested sampling design. Each of five populations was collected in environments that were as similar as possible and comprised a group representative of one of the following environments/vegetation communities (in order of increasing mean annual rainfall and predictability): desert, semisteppe batha, Mediterranean grassland and mountain (D1-5, B1-5, G1-5 and M1-5, respectively – Figure 1). Relief,
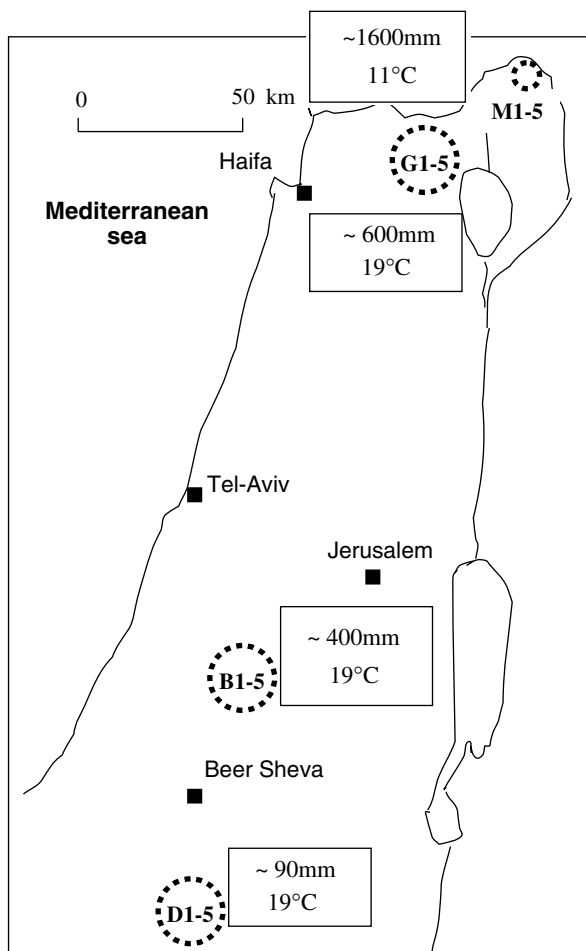
Figure 1 Mean annual rainfall (mm), annual temperature and geographic distribution of sampling localities of wild barley in Israel: M1-5 = mountain; G1-5 = grassland; B1-5 = semisteppe batha; D1-5 = desert.

slope exposition, vegetation and soil types were kept constant in sampling localities within a group (for a detailed description of the environments, see Volis *et al*, 2002d). Populations comprising a group were ≤5 km (mountain) or ≤20 km (other three groups) from each other. All 20 populations were used in a study of population genetic structure, and one (pivot) population from each group was used in a comparative study of plant life histories and in a test of local adaptation (Volis *et al*, 2002d). Environment-specific (region-specific) natural selection was explicit and detected *de facto* by reciprocal transplants of seeds and seedlings (Volis *et al*, 2002d). Significant genotype-by-environment interactions and highest fitness of genotypes in indigenous environments (except batha genotypes that had highest fitness in the grassland transplant site) were found, indicating local adaptation. Clear evidence of local adaptation was also demonstrated for two ecotypes, desert and batha, in an earlier study (Volis *et al*, 2002b).

Quantitative traits

One mature spike per plant was collected in 1996 from approximately 50 plants in each of 20 populations. From all populations, the seeds from nine random chosen spikes (one seed per spike) were sown in a greenhouse. After 3 weeks, when plants were at the 2–3 leaf stage, they were transferred to 10 l buckets filled with a potting mixture of equal parts peat and vermiculite with a single plant/pot. Buckets were placed in a nethouse at the Institutes for Applied Research (Beer Sheva, Israel) that contained drip lines with 2 l/h drippers with one dripper placed in each bucket. A randomized block design was used with a block containing 20 plants (one plant per population). Owing to some mortality during the experiment, the number of plants per population ranged from six to nine plants, and number of plants per region from 36–45 plants.

In a second common garden experiment, we used the progeny of eight randomly chosen F1 plants per population. The number of populations was four, with one (pivot) population per region. Prior to the experiment, the mother plants were planted under uniform conditions in a greenhouse and four offspring per mother plant were used in the experiment. As wild barley is predominantly autogamous, the offspring of each mother plant can be considered as a genetically identical single genotype. Each of four offspring was randomly assigned to one of four blocks containing 32 plants.

The common garden experiments were conducted at the same time and the same set of quantitative traits was measured. After the appearance of reproductive tillers, the first three reproductive tillers of each plant were tagged with colored tags representing the first, second and third tiller in order of awn appearance and each tiller was measured for: number of spikelets per spike (NSP), tiller height (TH), flag and penultimate leaf length and width (FLL, PLL, FLW and PLW), spike length (SPL), awn length (AWL), number of nodes (NN), and the number of days to awning (DAW) were recorded. At senescence, mean spikelet weight (SWT) was obtained from the three tagged spikes.

Allozymes and RAPDs

Nine enzyme systems encoding 15 loci were examined in 25 plants per population by starch gel electrophoresis

(for details see Volis *et al*, 2003). RAPD analysis was performed using DNA from 14 plants per population as described in Volis *et al* (2001).

Quantitative genetic analyses

The structure of variation in phenotypic traits was analyzed after running nested ANOVA by partitioning the total variance among regions (df 3), among populations within a region (df 16) and among individuals within a population (df 120–180) (common garden 1) or among populations (df 3), among genotypes within a population (df 28) and within genotypes (df 96) (common garden 2). In common garden 1, variance was also partitioned into among- and within-population components for each region separately.

The data from common garden 1 (20 populations) allowed us to determine the among-population (σ^2_{Pop}) and among-region (σ^2_{Reg}) genetic variance, and to estimate regional subdivision Q_{RT} and population subdivision Q_{ST} as $V_{GB}/(V_{GB} + V_{GW})$, where V_{GB} is the proportion of genetic variance distributed either among populations (σ^2_{Pop}) or among regions (σ^2_{Reg}) and V_{GW} is the proportion of genetic variance distributed within populations (Spitze, 1993; Morgan *et al*, 2001). The population subdivision within each region (Q_{SR}) was analyzed by conducting nested analysis of variance for each region separately. As genotypes were not replicated in the 20 population experiment, the within- and among-population components comprised both genetic and pure environmental components. However, we consider such Q_{ST} a useful surrogate for true Q_{ST} (with only a slight upward bias) because the environmental component for all traits except YLD was small and broad-sense heritability was very high (as revealed in the four population experiment; Table 2). For individual trait Q_{RT} values, the 95% confidence limits were calculated, adopting the equation given in Lynch and Walsh (1998) as

$$\frac{(F/F_U) - 1}{(F/F_U) + n - 1} < Q_{RT} < \frac{(F/F_L) - 1}{(F/F_L) + n - 1}$$

where F, F_U and F_L are the observed, upper and lower F values, respectively, associated with ($\alpha/2$) at ($N-1$), ($T-N$) degrees of freedom. For the average Q_{RT} and Q_{SR}, the 95% confidence intervals were estimated by bootstrapping with random reassignment to regenerate the original sample sizes repeated 1000 times. This was done using the program RESAMPLING STATS (Simon, 1995).

Data obtained in common garden 2 (four populations) were used to estimate broad-sense heritability, $h^2 = V_G/(V_G + V_E)$. One-way ANOVA was performed for each of the four populations. The within-genotype variance (σ^2_E) estimated the environmental variance V_E and the among-genotype variance provided an estimate of the genetic variance V_G. The standard errors of trait h^2 were calculated following Lynch and Walsh (1998) as $(1 - h^2)[1 + (n - 1)h^2]\sqrt{2/[Nn(n - 1)]}$, where N is the number of genotypes and n is the number of individuals per genotype.

The amount of trait-specific regional variance was expressed as the coefficient of variation $CV = \sqrt{\sigma^2_{\text{Reg}}}/X$, where X is the regional phenotype mean, and compared across regions by Levene's Test (JMP, 1995). The regional means were compared by using Welch ANOVA which allows variances to be unequal. The region differences over all traits considered simultaneously were analyzed by a single factor MANOVA followed by pairwise group contrasts. All the analyses of variance were done using JMP version 3.1.2 statistical package (JMP, 1995).

Principal component analysis was conducted on standardized original values using the Multi-Variate Statistical Package (MVSP, Kovach, 1999). A matrix of pairwise Euclidean distances between 20 populations was calculated by NTSYSpc program (Rohlf, 1998) and used in a Mantel test against RAPD and allozyme genetic distance matrices. Evaluation of significance of the matrix correlation employed a comparison of the observed Mantel test statistic, Z, with its random distribution generated by 1000 random permutations after the sequential Bonferroni adjustment for multiple testing.

Molecular genetic analyses

For both RAPDs and allozymes, the distribution of genetic variability within and among populations and regions was investigated by an analysis of molecular variance (AMOVA; Excoffier *et al*, 1992) and using Nei's G_{ST} statistic (Nei, 1973). For the former, genetic distances between haplotypes were calculated based on squared Euclidean distances. As allozymes showed very low observed heterozygosity ($<1\%$) (Volis *et al*, 2003), the heterozygotes were excluded and the data treated as haplotypes. For the latter, allele frequencies calculated from the RAPD bands assumed full dominance. Analyses were carried out for the whole sample and for each region separately. The number of permutations for significance testing was set at 1000 for analysis. The G_{ST} statistic was calculated with POPGENE version 1.31 (Yeh *et al*, 1998). This program was also used to calculate the pairwise population genetic distances (Nei, 1978) used in a Mantel test against each other and genetic distances calculated from quantitative traits. Principal component analysis was used to ordinate relationships among individuals and among regions using the MVSP program.

Results

Quantitative genetic variation

Regional means and coefficients of variation for 11 quantitative traits are shown in Table 1. Regional values differed significantly in each trait (Welch ANOVA) and over all traits analyzed simultaneously (Wilks' $\lambda = 0.04$, $F_{33,430}$ 26.8, $P < 0.001$, MANOVA). MANOVA also revealed highly significant differences ($P < 0.001$) in each pairwise region comparison. Variation within groups, although different for some traits (as revealed by Levene's test), showed no consistent pattern across the groups. In all traits, we found a trend of either an increase or decrease in trait values from desert to grassland, with plants from the mountain region strongly resembling those from the grassland (Table 1 and Figure 2). Heritability of all traits except YLD was high, ranging 0.33–0.94 when averaged over four populations, but with striking differences among populations

Table 1 Regional means and coefficients of variation (in parentheses) for 11 quantitative traits

Region	n	DAW	NSP	SWT	YLD	SPL	AWL	FLL	FLW	PLL	PLW	TH
Desert	36	120.0 (3.8)	37.4 (8.6)	48.6 (14.6)	2884 (26.3)	20.3 (7.9)	6.0 (16.7)	13.4 (24.6)	1.09 (15.6)	26.8 (18.7)	1.51 (12.6)	63.9 (13.1)
Batha	44	131.4 (3.9)	33.9 (8.8)	70.3 (18.9)	2444 (20.8)	24.3 (7.0)	10.1 (16.8)	9.2 (27.2)	0.90 (20.0)	17.8 (20.2)	1.42 (21.8)	77.6 (11.2)
Grassland	45	140.3 (3.6)	29.1 (7.9)	79.4 (13.6)	2438 (18.8)	28.6 (11.9)	15.2 (22.4)	6.0 (23.3)	0.53 (22.6)	13.2 (17.4)	1.00 (15.0)	85.7 (13.0)
Mountain	39	145.3 (2.8)	31.6 (9.5)	70.4 (11.2)	2498 (16.8)	28.9 (11.1)	14.0 (20.0)	7.2 (26.4)	0.51 (21.6)	14.8 (16.9)	0.89 (18.0)	87.4 (9.6)
Levene's test		0.94 ns	1.95 ns	3.8*	4.0**	12.5***	15.6***	8.0***	2.9*	10.3***	8.3***	1.7 ns
Welch's ANOVA		234.1***	62.8***	96.0***	3.4*	118.0***	179.0***	62.2***	57.8***	81.8***	96.7***	56.4***

Level of significance: * – 0.05; ** – 0.01; *** – 0.001; ns = not significant.
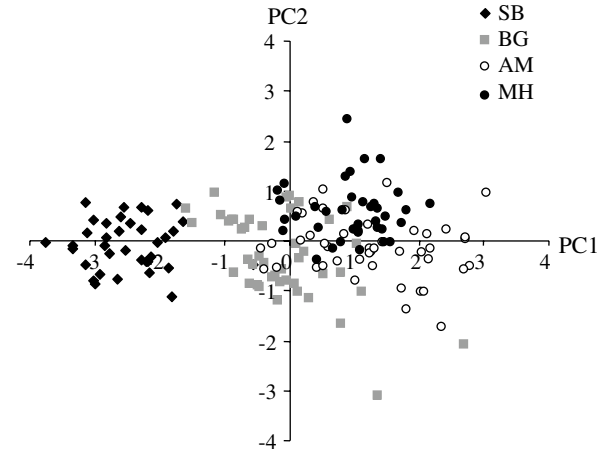
Figure 2 Plot of principal components for quantitative trait variation.

(Table 2). Average trait h^2 was significantly higher in the desert population than in batha and mountain populations (Wilcoxon Signed-Ranks Test, $Z = 2.49$ and 1.96, $P < 0.05$), and higher in the grassland population than in batha (Wilcoxon Signed-Ranks Test, $Z = 2.40$, $P < 0.05$). The traits can be divided into two groups on the basis of their intrapopulation differences in heritability: (1) with uniformly high (> 0.8) or low (< 0.3) h^2 (SPL, AWL and YLD); (2) with highly variable h^2 among populations (remaining traits).

Partitioning of variation in quantitative traits revealed a very high regional component and regional subdivision Q_{RT} ranged from 0.55 to 0.89 for all traits except one (YLD) (Table 2). Regions contributed more to the total variation than populations, genotypes or environment altogether in all traits except YLD and TH. Subdivision among populations within regions averaged over traits ranged from 0.17 to 0.40 (Table 3).

Molecular variation for RAPDs and allozymes

Total and within-population gene diversity estimates (H_T and H_S) for RAPDs and allozymes averaged across loci did not differ, nor did four regional estimates (Table 3). Despite the fact that the patterns of differentiation in RAPDs and allozymes matched in general, the allozymes exhibited lower regional subdivision than RAPDs (Table 3), and most of this variation appears to be due to segregation of the mountain population from the rest (not shown), with variation in allozymes being much more discontinuous than in RAPDs. The latter appears to be due to a limited number of polymorphic allozyme loci (only five loci exhibited $H_T > 0.3$). The mountain region was clearly distinct for both marker types but segregation of the other three regions was less obvious, especially for allozymes (not shown). The levels of regional subdivision and population subdivision within regions were similar for both types of markers with high agreement between the estimates of subdivision by AMOVA and Nei's gene diversity (Table 3).

Relationships between molecular and quantitative trait variation

A test of the relationships between three matrices of pairwise genetic distances (quantitative trait values,

Table 2 Estimates of variance components (in %), regional differentiation (Q_{RT}) and heritability (h^2) for 11 quantitative traits

Trait	20 populations experiment				4 populations experiment						
	Variance components			Q_{RT}	Variance components			h^2			
	σ^2_{Reg}	σ^2_{Pop}	σ^2_{within}		$\sigma^2_{Reg+Pop}$	σ^2_{Gen}	σ^2_{E}	Desert	Batha	Grassland	Mountain
DAW	81.4	9.3	9.3	0.897 (0.857–0.965)	62.9	34.4	2.7	0.972 (0.008)	0.689 (0.069)	0.830 (0.043)	0.797 (0.050)
NSP	53.8	17.1	29.1	0.649 (0.531–0.856)	32.4	52.7	14.9	0.725 (0.063)	0.492 (0.091)	0.795 (0.050)	0.912 (0.024)
SWT	56.1	10.8	33.1	0.629 (0.492–0.838)	70.8	8.7	20.5	0.778 (0.053)	0.118 (0.086)	0.427 (0.094)	−0.017 (0.070)
YLD	4.8	18.7	76.5	0.059 (−0.186–0.142)	28.4	21.2	64.5	0.022 (0.075)	0.077 (0.082)	0.242 (0.094)	0.105 (0.085)
SPL	65.5	17.4	17.1	0.793 (0.722–0.926)	77.2	18.7	1.6	0.973 (0.008)	0.938 (0.017)	0.817 (0.045)	0.921 (0.022)
AWL	68.5	17.0	14.5	0.825 (0.762–0.938)	80.2	43.7	1.0	0.985 (0.004)	0.961 (0.011)	0.924 (0.020)	0.900 (0.027)
FLL	60.6	6.0	33.4	0.645 (0.514–0.848)	43.0	20.0	13.2	0.813 (0.046)	0.403 (0.095)	0.807 (0.048)	0.262 (0.095)
FLW	76.6	5.9	17.5	0.814 (0.738–0.931)	64.4	37.4	15.5	0.698 (0.067)	0.211 (0.093)	0.603 (0.080)	−0.011 (0.071)
PLL	72.1	2.7	25.2	0.741 (0.640–0.898)	35.0	18.7	27.5	0.802 (0.049)	0.224 (0.094)	0.176 (0.091)	0.523 (0.088)
PLW	61.8	17.2	21	0.746 (0.658–0.904)	65.2	11.0	16.1	0.512 (0.089)	0.517 (0.089)	0.684 (0.070)	0.118 (0.086)
TH	47.8	13.2	39	0.551 (0.401–0.794)	80.9	7.0	8.0	0.661 (0.073)	0.156 (0.089)	0.574 (0.084)	0.737 (0.061)
Mean CI 95% (bootstrapped over traits)	59.0	12.3	28.7	0.668 (0.471–0.757)	58.2	24.9	16.9	0.722 (0.495–0.840)	0.435 (0.277–0.645)	0.626 (0.462–0.755)	0.477 (0.249–0.691)

Variance components: σ^2_{Reg} = among-region; σ^2_{Pop} = among-population; σ^2_{Gen} = among-genotype; σ^2_{E} = environmental; σ^2_{within} = within population including environmental; $\sigma^2_{Reg+Pop}$ = among-region including among-population component.

Table 3 Molecular and quantitative trait subdivision estimated with θ-statistics (AMOVA; Excoffier *et al*, 1992), G_{ST}-statistics (Nei, 1973) and Q_{ST}-statistics (Spitze, 1993). Estimates of θ_{ST} and G_{ST} (RAPDs and allozymes) and Q_{ST} (quantitative traits) are presented as both regional subdivision (θ_{RT}, G_{RT} and Q_{RT}) and population subdivision within regions (θ_{SR}, G_{SR} and Q_{SR}). Estimates of H_T, Q_{RT} and Q_{SR} are provided with 95% CI (bootstrapping over loci/traits)

	RAPDs					Allozymes					Quantitative	
	θ_{RT}	θ_{SR}	H_T	G_{RT}	G_{SR}	θ_{RT}	θ_{SR}	H_T	G_{RT}	G_{SR}	Q_{RT}	Q_{SR}
Total	0.205	0.256	0.253 (0.223–0.285)	0.207	0.311	0.162	0.096	0.224 (0.154–0.294)	0.108	0.112	0.628 (0.471–0.757)	0.324 (0.239–0.417)
Desert		0.219	0.200 (0.169–0.247)		0.231		0.041	0.217 (0.163–0.276)		0.064		0.169 (0.114–0.256)
Batha		0.166	0.207 (0.169–0.239)		0.175		0.092	0.193 (0.138–0.255)		0.086		0.261 (0.152–0.385)
Grassland		0.378	0.202 (0.162–0.238)		0.355		0.144	0.207 (0.129–0.286)		0.168		0.274 (0.136–0.404)
Mountain		0.509	0.194 (0.156–0.244)		0.494		0.177	0.184 (0.114–0.262)		0.134		0.396 (0.254–0.559)

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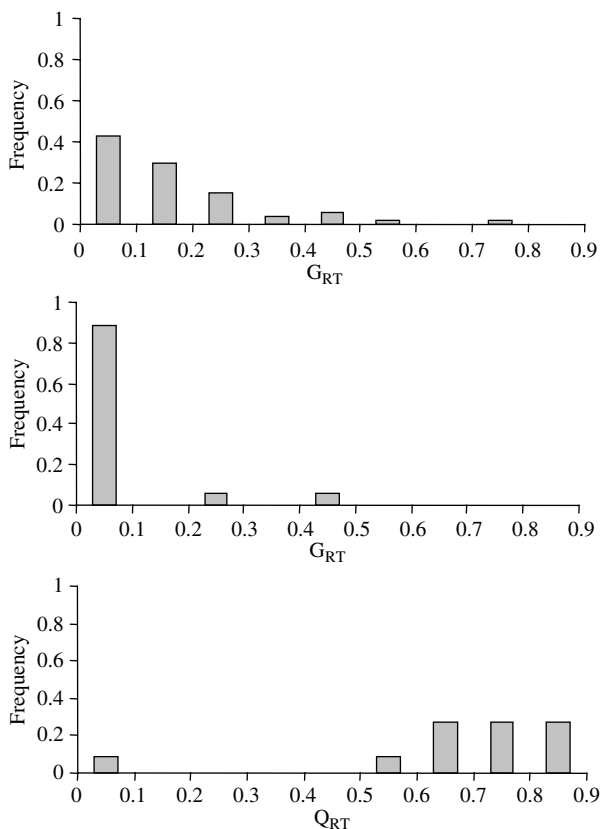
Figure 3 Histograms of regional differentiation estimates for 54 RAPD loci (G_{RT}), 17 allozyme loci (G_{RT}) and 11 quantitative traits (Q_{RT}) (from top to bottom).

allozyme and RAPD allele frequencies) calculated from 20 populations revealed the following: There was a highly significant relationship between allozyme and RAPD Nei's genetic distances ($r = 0.69$, $P < 0.001$, Mantel test), but not between quantitative traits and either allozymes and RAPDs ($r = 0.02$ and 0.26, $P > 0.05$, Mantel's test). The pairwise estimates of population subdivision θ_{ST} for two molecular marker types were also highly correlated ($r = 0.70$, $P < 0.001$, Mantel test) with one another. The within-population levels of genetic variation for quantitative traits (CV), allozymes and RAPDs H_E were not correlated with one another. The within-population gene diversity estimated with allozymes (mean = 0.173, range 0.114–0.253) was higher than that estimated with RAPDs (mean = 0.138, range 0.040–0.202) (Wilcoxon Signed-Ranks Test, $Z = 2.65$, $P = 0.008$).

The frequency distribution of regional subdivision estimates was skewed in opposite directions for quantitative traits (mode $0.6 < Q_{RT} < 0.9$) vs allozyme and RAPD loci (mode $0 < G_{RT} < 0.1$) (Figure 3). The allozyme loci G_{RT} values, however, were almost exclusively located near the mode, while RAPD G_{RT} estimates varied widely.

Discussion

Quantitative genetic variation
Levels of regional subdivision for quantitative traits (Q_{RT}) were very high (0.55–0.90 in 10 out of 11 traits). These traits were previously analyzed for phenotypic plasticity (Volis et al, 2002e) and involvement in the adaptative process (Volis et al, 2004), including involvement in region-specific adaptation (Volis et al, 2002c), using plants from one (pivot) population per region. In these experiments, DAW, SPL, SWT, AWL and TH were significant predictors of individual plant fitness. Leaf dimensional traits (FFL, FLW, PLL, PLW) have also shown clear regional differences, apparently indicating region-specific adaptation (Volis et al, 2002e). The results reported on here were obtained using plants from 20 populations and are in full agreement with those previously derived from pivot populations only. Together, they suggest a region-specific adaptive process in wild barley that is detectable with quantitative traits. There is directional selection in H. spontaneum along a gradient of increasing aridity (from mountain to desert) for early transition to reproduction, and along the same aridity gradient from grassland to desert for increase in size of flag and penultimate leaves and number of seeds per spike, and a decrease in seed weight and size of reproductive structures. The plants from mountain experience specific environmental conditions (longer vegetative period and much colder winter with occasional frosts) but adaptation to these conditions may be achieved by physiological and not morphological traits, as the latter were very similar to those of grassland plants.

Regional genetic differentiation, both clinal and non-clinal, observed in studied quantitative traits is clearly adaptive after several reciprocal introductions performed either at two (Volis et al, 2002b) or four (Volis et al, 2002d) sampling locations. However, Verhoeven et al (2004), who demonstrated local adaptation to different nutrient environments in wild barley, failed to detect diversifying selection on the underlying QTL loci. The results of Verhoeven et al (2004) show that the common view that adaptive differentiation involves a genetic trade-off between alleles of the same locus that have opposite fitness effects in contrasting environments needs to be reconciled with an alternative view that adaptive differentiation is caused by loci that differ in the magnitude but not in the direction of their fitness effects across habitats (Kawecki, 1997).

Molecular variation for RAPDs and allozymes and relationship between markers
It has been predicted that the extent of genetic variation in quantitative traits and molecular gene diversity (expressed as expected heterozygosity H_E) are correlated (Falconer and Mackay 1996). This, however, should be true only if the variation is neutral, because such forces as drift and migration will similarly affect neutral but not fitness-related loci (Lewontin and Krakauer, 1973; Slatkin, 1987). As the quantitative traits examined in this study were found to be adaptive, it is not surprising that we failed to detect a relationship between either allozyme or RAPD H_E and variation in any of the quantitative traits. A lack of correlation between within-population allozyme and quantitative variation was also found in two other studies performed on wild plant species (Waldmann and Andersson, 1998; Podolsky, 2001).

Our results indicate that there is a positive relationship between interpopulation genetic distances obtained from

allozyme- and RAPD-based data, which is consistent with the results of many other studies (eg Heun *et al*, 1994; Lifante and Aguinagalde, 1996; Wu *et al*, 1999; Bartish *et al*, 2000a). However, we did not find a positive relationship between corresponding estimates of within-population variation. In contrast to generally observed similarities in allozyme- and RAPD-generated estimates of within-population variation or higher estimates for RAPDs (reviewed in Vandewoestijne and Baguette, 2002; Nybom, 2004), we found the opposite pattern. Higher regional and population subdivision for RAPDs *vs* allozymes contrasted with lower within-population variation for RAPDs. One possible explanation could be a smaller sample size for RAPDs *vs* allozymes (14 and 25 plants per population, respectively).

Patterns of inter-regional variation for quantitative traits and the two molecular markers visualized by principal components analysis were not congruent. However, allozyme and RAPD scatterplots showed more in common than did a scatterplot of quantitative traits with either of the other two (in particular, both molecular marker types, but not quantitative traits, revealed a segregation of the mountain region from the other three regions). Additionally, matrices of allozyme and RAPD pairwise population genetic distances were significantly correlated with each other but not with a matrix for quantitative traits. As quantitative traits were found to be adaptive, this implies that most allozyme and RAPD variation is nonadaptive.

The degree of regional subdivision for allozymes and RAPDs was lower than for quantitative traits. The level of regional subdivision in two molecular markers was comparable with population subdivision within a region, while in quantitative traits it was significantly (almost $2\times$) higher. Disagreement between the structure of variation at two scales (regions and populations within regions) in quantitative traits and two molecular markers, and the pattern of molecular variation reported here and in Volis *et al* (2001, 2003) point at selective neutrality of overall allozyme and RAPD variation. However, effects of selection or gene hitch-hiking on allozyme and RAPD variation cannot be ruled out completely. The frequency distributions of G_{RT} values of allozyme and RAPD loci show that most loci exhibit minor or no regional differentiation, and that the relatively high overall estimates of G_{RT} are due to several loci with exceptionally high regional differentiation. This pattern is difficult to explain by genetic drift alone as all neutral loci are expected to be affected in the same manner. Natural selection, operating on these exceptional loci, is a more plausible scenario. However, although this scenario is plausible, it still needs to be supported by experiments. An alternative explanation of nonuniformity of genetic subdivision across loci is that the selective process has an effect on those neutral loci that are in linkage disequilibrium with loci directly affected by selection (Maynard Smith and Haigh, 1974). This stems from the theory of hitch-hiking that, within a single population and under homogeneous environmental conditions, reduced gene diversity is expected at a neutral locus closely linked to the selected locus (Maynard Smith and Haigh, 1974; Barton, 1995). However, in a geographically subdivided population (Slatkin and Wiehe, 1998) or under local adaptation (Charlesworth *et al*, 1997), hitch-hiking may lead to high G_{ST}

values even in the case of significant gene flow, and despite simultaneous reduction in within-population gene diversity.

In conclusion, the results of this study showed a moderate to high degree of differentiation among populations of wild barley in both molecular (allozymes and RAPDs) and quantitative traits. However, examination of population subdivision at two spatial scales, one of which was ecologically relevant, revealed two different outcomes. The Q_{ST} and F_{ST} did not differ at the population scale, that is, within regions, where stochastic evolutionary forces appear to prevail, but differed at the regional scale, where an adaptive differentiation process was previously found to occur. This implies that the comparison of levels of subdivision in quantitative traits (most of which are closely related to fitness) with molecular markers (which fitness effects are unknown) is indicative of adaptive population differentiation only when sampling is done at the proper scale.

Acknowledgements

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