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Genetic diversity and ecogeographical differentiation among ribosomal DNA alleles in wild and cultivated barley

(restriction fragment length variants/Mendelian ribosomal DNA loci/selection/adaptedness)

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ABSTRACT **DNA from 267 accessions of wild barley from ecologically diverse habitats in Israel and Iran and from 92 accessions of cultivated barley from throughout the world were assayed for the 20 ribosomal DNA (rDNA) spacer-length variants that have been identified in the barley species. These 20 spacer-length variants, which are detectable by Southern blot hybridization, serve as markers of rDNA alleles of two Mendelian loci, *Rrn1* and *Rrn2*. All of the populations of wild barley studied were polymorphic for both loci. In wild barley allele 112 (*Rrn1*) and allele 107 (*Rrn2*) behaved as widely adapted wild-type alleles; in our sample of cultivated barley allele 112 also behaved as a wild-type allele but allele 104 was somewhat more frequent than allele 107 in *Rrn2*. A few other alleles were locally frequent in wild barley. However, most of the 20 alleles were infrequent or rare and such alleles were often associated as "hitchhikers" with one of the wild-type alleles in compound two-component alleles. Allelic and genotypic frequencies differed widely in different habitats in correlation with eight of nine factors of the physical environment. Discrete log–linear multivariate analyses revealed statistically significant associations among alleles of *Rrn1* and *Rrn2*. It was concluded that natural selection acting differentially on various rDNA alleles plays a major role in the development and maintenance of observed patterns of molecular and genetic organization of rDNA variability.**

Hordeum vulgare ssp. *spontaneum* (*H.S.*), the wild ancestor of cultivated barley [*Hordeum vulgare* ssp. *vulgare* (*H.V.*)], occurs in a wide range of ecologically diverse habitats in Southwest Asia. *H.V.*, which is confined to arable fields in which conditions of life differ strikingly from those in the wild, occurs under a narrower range of climatic and edaphic conditions. Numerous studies have established that barley, although predominantly self-fertilizing (≈99%), is polymorphic for many discretely inherited morphological, physiological, allozyme, and DNA variants and that this variation is correlated with numerous physical and biotic features of the environment (for review, see ref. 1). Herein, we report the distribution and frequencies of 20 ribosomal DNA (rDNA) alleles of Mendelian loci *Rrn1* and *Rrn2* (2, 3) in populations of *H.S.* from Israel and Iran and in a worldwide sample of *H.V.* Our results show that *H.S.* is substantially more variable than cultivated barley and that specific alleles of the two rDNA loci are associated with each other and with specific factors of the physical environment.

MATERIALS AND METHODS

Genetic Materials. The genetic materials of this study were 238 accessions of *H.S.* from Israel, 29 accessions of *H.S.* from Iran, and 92 accessions of *H.V.*, representing all of the major barley growing areas of the world. All 359 accessions were from the USDA world barley collection. Single plants of each accession were scored for presence or absence of specific rDNA spacer-length variants (slvs). The 161 individuals of sample I were from 14 locations in Israel (see figure 1 of ref. 4). The exact locations in which the 77 remaining accessions from Israel were collected are unknown; the 77 individuals scored from these accessions were designated sample II. The 29 individuals from four locations in Iran were designated sample III and the 92 individuals of *H.V.* were designated sample IV. Data on nine factors of the physical environment were available for the 14 sites of sample I and the four sites of sample III; these data were utilized in multivariate log–linear analyses of the distribution of allelic and genotypic variability in different habitats.

DNA Preparation and Detection of rDNA Alleles. DNA preparation, electrophoresis, and hybridization closely followed described procedures (2). A genomic clone, pTA71, which contains the entire wheat rDNA repeat unit, was used as the hybridization probe (5). The rDNA of plants is arranged in tandemly repeating arrays in which each repeat consists of a transcription unit and an intergenic spacer (IGS) region (for reviews, see refs. 6 and 7). In barley, restriction enzyme *Sst* I cleaves each of the rDNA units twice, once on each side of the IGS region, thus generating two rDNA fragments from each repeat unit (2). One fragment, made up primarily of the transcription unit, is invariant [≈3880 base pairs (bp)] whereas the other fragment, which represents the major portion of the IGS region, varies in length as a result of differences in the number of subrepeats present. Twenty of these *Sst* I length-variable fragments, referred to as rDNA slvs, have been identified in barley by Southern blot analysis (2, 3). Each variant differs in length from the immediately adjacent variants by 115 bp so that the series forms a complete ladder; the shortest variant, which lies in an *Sst* I fragment 4625 bp long, is designated slv 100 and the longest variant, which lies in an *Sst* I fragment 6595 bp long, is designated slv 118. A single exceptional variant, slv 108a, is ≈42 bp shorter than slv-108. Analyses of Mendelian segregation ratios (2, 3) in F_2 families have established that slvs 100–107 segregate as codominant alleles of Mendelian locus *Rrn2*, associated with the nucleolar organizer region of chromosome 7, and that slvs 108a–118 segregate as codominant alleles of Mendelian locus *Rrn1*, associated with the nucleolar organizer region of chromosome 6. Thus the 20 slvs

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Abbreviations: rDNA, ribosomal DNA or rRNA gene; *H. S.*, *Hordeum vulgare* ssp. *spontaneum*, wild barley; *H.V.*, *Hordeum vulgare* ssp. *vulgare*, cultivated barley; sl, spacer length; slv(s), spacer-length variant(s); IGS region, intergenic spacer region.

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of barley are organized in two families, one forming a regularly complete 8-step ladder of variants from 4625 to 5430 bp long and the other forming a 12-step ladder of variants from 5545 to 6695 bp long (≈42 bp shorter when slv 108a is present). The eight shorter slvs (slvs 100–107) serve as markers of 8 alleles (100–107) of Mendelian locus *Rrn2* and the 12 longer slvs (108a–118) serve as markers of the 12 alleles of Mendelian locus *Rrn1*. Each of the 20 alleles is composed of an array of numerous tandem repeats of a transcription unit ≈3880 bp long plus its associated IGS region from ≈4625 to 6595 bp long (2, 3). Extensive surveys of IGS variability in barley have shown that individual plants are typically homozygous for two pairs of rDNA alleles, including one pair representing *Rrn1* and one pair representing *Rrn2*.

RESULTS

Phenotypic (Genotypic) Variation in *H.S.* and *H.V.* Numbers and frequencies of rDNA spacer-length (sl) phenotypes (genotypes) observed in samples I, II, III (*H.S.*), and IV (*H.V.*) are given in Table 1. Forty-two phenotypes were observed in total, 36 in *H.S.* and 10 in *H.V.* (4 were common to *H.S.* and *H.V.*). Thus, *H.S.* was clearly more variable than *H.V.* for the rDNA alleles marked by the slvs.

Among the 359 individual plants assayed, 314 (87%) had two slvs and, among these 314 individuals, 297 (95%) were typical in that they had one slv from series 100–107 and one from series 108a–118. The 17 remaining individual plants (5%) with two slvs were atypical in that their slvs were both from series 100–107 or both from series 108a–118. Seven among the 359 plants (2%) had only one slv; 5 of these plants were phenotypically 112 (genotypically, 112,112, 112,112) and two were phenotypically 107 (genotypically, 107,107 107,107). Four among the 359 plants (1%) had four slvs; these plants were either doubly heterozygous first-generation natural hybrids or descendents of recent natural hybrids between pairs of parental plants with four slvs in total (3). Thirty-four among the 359 plants (9%) had three slvs and hence they were either singly heterozygous natural hybrids (or descendents of recent natural hybrids) or they were true breeding for one normal allele and for one compound allele marked by two slvs (3). Other studies (R.W.A., unpublished data) indicate that about half of the plants with three slvs are single heterozygotes and half carry a compound allele.

Table 1. sl phenotypes (genotypes) observed in samples I, II, and III (*H.S.*) and sample IV (*H.V.*)

sl phenotype	*n* per sample			Total I–III		Sample IV	
	I	II	III	*n*	*f*	*n*	*f*
107,112	66	15	21	102	0.38	19	0.21
104,112	1	0	1	2	<0.01	56	0.61
107,108	14	14	0	28	0.10	0	0.00
107,109	20	4	0	24	0.09	0	0.00
106,108	12	2	0	14	0.05	0	0.00
107,108a	3	8	2	13	0.05	0	0.00
106,112	9	2	0	11	0.04	0	0.00
107,110	7	3	0	10	0.04	0	0.00
105,107	1	9	0	10	0.04	0	0.00
100,107,112	6	0	0	6	0.02	2	0.02
106,109	3	2	0	5	0.02	0	0.00
112	0	0	0	0	0.00	5	0.05

Phenotypes present in $f > 0.01$ in the total of the four samples are given in the body of the table. Phenotypes present in $f < 0.01$ are as follows (numbers of phenotypes observed in parentheses). Additional phenotypes observed in sample I: 103,112 (1); 106,110 (2); 107,111 (1); 107,113 (1); 107,116 (1); 111,112 (2); 104,107,108 (2); 104,107,109 (1); 104,107,112 (3); 106,107,112 (2); 106,108,109 (1); 107,108,112 (1); 107,108,117 (1). Additional phenotypes observed in sample II: 102,107 (2); 104,110 (1); 105,112 (1); 107,111 (2); 108,109 (3); 103,107,112 (1); 104,107,110 (1); 105,107,108a (1); 105,107,112 (2); 107,108,112 (2); 107,108a,112 (1); 106,107,109,112 (1). Additional phenotypes observed in sample III: 107,113 (1); 107,108,112 (1); 107,112,114 (2); 103,107,112,114 (1). Additional phenotypes observed in sample IV: 107 (2); 102,112 (1); 107,111,112 (1); 101,104,112 (3); 104,107,112 (1); 106,107,111,112 (2).

Allelic Variation in *H.S.* and *H.V.* Table 2 gives numbers and frequencies of alleles of *Rrn1* and *Rrn2* observed in samples I–IV. Two conventions were followed in assigning alleles to loci and in enumerating numbers of alleles: (*i*) All alleles in the series 100–107 were assigned to *Rrn2* and all alleles in the series 108a–118 were assigned to *Rrn1*. Thus, all four alleles of plants with phenotypes such as 107,105 were assigned to *Rrn2* even though Mendelian analyses (3) have shown that either allele 105 or 107 is resident in *Rrn1* in such plants; consequently, the numbers of alleles assigned to *Rrn1* and *Rrn2* in Table 2 were not necessarily the same. (*ii*) All plants with three slvs were considered to be heterozygous at one locus and homozygous at the other locus; this convention leads to slight underestimation of the frequencies of alleles marked by compound slvs.

It can be seen from Table 2 that allele 107 was the most common allele of *Rrn2* in *H.S.* [frequency (f) = 427/548 = 0.78]. Allele 107 was followed in frequency by alleles 106 (0.13), 105 (0.05), 104 (0.02), and 100 (0.01), and two rare alleles (102 and 103). In *H.V.*, allele 104 (0.65) was the most common allele of *Rrn2*, followed by alleles 107 (0.30), 106 and 101 (0.02), and 102 and 100 (0.01).

Table 2 also shows that allele 112 (0.53) was the most common allele of *Rrn1* in *H.S.*, followed by alleles 108 (0.19), 109 (0.13), 108a (0.06), 110 (0.05), and 111 (0.02), and four rare alleles (117, 116, 115, 113). Locus *Rrn1* was much less variable in *H.V.* than in *H.S.*; only two alleles were observed in *H.V.*, alleles 112 (0.98) and 111 (0.02).

Genotypic Variation in rDNA Within Populations. Genotypic variation within populations was estimated from individuals collected from 14 sites in Israel (sample I) and from four sites in Iran (sample III). The mean number of individ-

Table 2. Alleles of *Rrn1* and *Rrn2* observed in samples I, II, and III (*H.S.*) and sample IV (*H.V.*)

slv	*n* per sample			Total I–III		Sample IV	
	I	II	III	*n*	*f*	*n*	*f*
Rrn2							
100	6	0	0	6	0.01	2	0.01
101	0	0	0	0	0.00	3	0.02
102	0	4	0	4	<0.01	2	0.01
103	2	1	1	4	<0.01	0	0.00
104	8	3	2	13	0.02	116	0.65
105	2	23	0	25	0.05	0	0.00
106	56	13	0	69	0.13	2	0.02
107	246	126	55	427	0.78	53	0.30
Total	320	170	58	548	1.00	178	1.00
Rrn1							
108a	6	19	4	29	0.06	0	0.00
108	59	40	1	100	0.19	0	0.00
109	49	19	0	68	0.13	0	0.00
110	18	10	0	28	0.05	0	0.00
111	6	4	0	10	0.02	3	0.02
112	181	44	48	273	0.53	187	0.98
113	2	0	2	4	<0.01	0	0.00
114	0	0	3	3	<0.01	0	0.00
115	0	0	0	0	<0.00	0	0.00
116	2	0	0	2	<0.01	0	0.00
117	1	0	0	1	<0.01	0	0.00
118	0	0	0	0	0.00	0	0.00
Total	324	136	58	518	1.00	190	1.00
Grand total	644	306	116	1066		368	

uals assayed per site was 10.6 (range 2–21). The mean number of genotypes observed per site was 3.55 (range 1–9). Genotypic diversity indices calculated using the Shannon information statistic ranged from 0.00 to 1.70 (mean, 0.88). Partitions of total genotypic diversity indicated that 35% was attributable to within- and 65% was attributable to among-population differences. The number of alleles of *Rrn1* per population ranged from 1 to 4 (mean = 2.38) and from 1 to 3 (mean = 1.94) for *Rrn2*. Allelic diversity indices ranged from 0.00 to 0.63 (mean = 0.34) for *Rrn1* and from 0.00 to 0.51 (mean = 0.17) for *Rrn2*. By considering both loci jointly, the number of alleles per population varied from 2 to 7 (mean 4.33) and allelic diversity indices varied from 0.00 to 0.50 (mean = 0.25). Partitions of total allelic diversity indicated that 47% was within and 53% was among populations. Thus, genotypic and allelic diversity were substantial both within and among nearly all populations. Genotypic diversity was, however, greater than allelic diversity; also diversity for *Rrn1* was somewhat greater than for *Rrn2*.

Genotypic Differentiation Among Populations. Table 3 gives the numbers of populations of *H.S.* (samples I and III) in which designated phenotypes (genotypes) were observed in locally high frequencies. Phenotype 112,107 (genotype, 112,112, 107,107) was predominant ($f > 0.50$) in 15 of the 18 populations. However, it was accompanied by other phenotypes (genotypes) in several populations, including 108,107 as a major secondary companion in three populations, 109,107 in two populations, and 110,107 in one population. Phenotypes 112,106, 108a,107, and 108,106 were each predominant ($f > 0.50$) in one population. Note that phenotype 108,106 is the only prominent phenotype that did not include allele 112, allele 107, or both alleles. It is apparent from the data of Table 3 that many of the populations of samples I and III are sharply differentiated genetically.

Associations Among sl Markers and Environmental Factors. Data were available for nine environmental factors for the sites from which the 18 populations of samples I and III were collected. The nine factors were humidity (designated *H*), altitude (*A*), evaporation (*E*), annual rainfall (*R*), mean annual temperature (*T*), mean January temperature (*J*), mean August temperature (*U*), latitude (*I*), and longitude (*O*). Discrete multivariate statistical techniques (8, 9) were employed in analyzing the interrelationships among the rDNA alleles and the environmental factors. These techniques provide tests for identifying and eliminating terms with statistically nonsignificant effects in each system and for constructing models that characterize associations among factors in the system. Models were constructed in a hierarchical manner such that a higher-order term was included only when lower-order terms failed to fit the data. When a higher-order term was included, all of its lower-order relatives were included. The "best-fitting" model was chosen on the basis that it was maximally parsimonius and also provided a statistically acceptable fit to the data. In applying these statistical techniques, the most frequent variants of *Rrn1* (112) and *Rrn2* (107) were designated allele 1 and the remaining alleles were combined to form composite "synthetic" alleles designated allele 2 for each locus. The nine environmental factors were divided into three disjunct classes representing three levels (low, medium, and high) for each factor. Contingency tables were constructed and log-linear techniques were used (9) to investigate associations among alleles of *Rrn1* and *Rrn2* and the environmental factors. We describe the analysis of relationships among *Rrn1* (*L*), *Rrn2* (*S*), and humidity (*H*), and among *L*, *S*, and altitude (*A*), to illustrate the statistical procedures used. Likelihood ratio tests and stepwise selection indicated that the model [*LH*] [*SH*] best fits the humidity data. Thus alleles of both *Rrn1* and *Rrn2* were associated with humidity but these two associations were independent of each other. The likelihood ratio statistic (G^2, 3 degrees of freedom) took value 3.96 with probability 0.27. The log-linear form of this model is

$$\ln m_{ijk} = u + u_{L(i)} + u_{S(j)} + u_{H(k)} + u_{LH(ik)} + u_{SH(jk)}$$

$$\text{for all } i, j = 1, 2 \text{ and } k = 1, 2, \text{ and } 3, \qquad [1]$$

in which m_{ijk} is the expected number of individuals with allele i of *Rrn1*, allele j of *Rrn2*, and belonging to humidity class k; $u_{L(i)}$, $u_{S(j)}$, and $u_{H(k)}$ are the effects of the ith allele of *Rrn1*, the jth allele of *Rrn2*, and the kth level of humidity; i.e., these terms compare the relative frequencies of these categories for each variable. The term $u_{LH(ik)}$ represents the association between the ith allele of *Rrn1* and the kth humidity level and $u_{SH(jk)}$ represents the association between the jth allele of *Rrn2* and the kth humidity level. Goodness of fit to the model [*LH*] [*SH*] was also checked in terms of standardized residuals. Table 4 shows observed and expected numbers and standardized residuals for each cell in the model [*LH*] [*SH*]. The largest absolute standardized residual value is 1.1, indicating a good fit to the model. The absolute values of the estimated standardized values for *u* (Table 5) are 3.31, 1.41, and 2.31 for the associations of *Rrn1* with low, medium, or high humidity levels (*LH* associations), whereas the values for the *SH* association are 2.62, 1.35, and 2.17, respectively. Thus, associations with humidity are such that alleles 1 of both *Rrn1* and *Rrn2* (alleles 112 and 107) occur more frequently in low humidity situations and alleles 2 of both loci occur more frequently in high humidity situations.

The saturated model [*LSA*], which features a third-order interaction, best fits the data set for altitude (*A*). Under this model expected and observed numbers in each of the 12 cells are equal and all standardized residuals are zero; the relationships among the two loci and altitude are, therefore, complex. Some of the estimated standardized *u* values (data not shown) were statistically significant for the *LA* and *SA*

Table 3. Populations of *H.S.* in which designated rDNA phenotypes (genotypes) were present in locally high frequency

rDNA phenotype		
Dominant	Secondary	Population(s), *n*
112,107		9
112,107	108,107	3
112,107	109,107	2
112,107	110,107	1
108,106		1
108a,107		1
112,106		1

For dominant phenotype, f is 0.5–1.0; for secondary phenotype, f is 0.20–0.40.

Table 4. Observed number, expected number, and standardized residual (top, middle, and bottom rows, respectively) for each cell under the model [*LH*] [*SH*]

		Rrn2-1			*Rrn2-2*		
Humidity	Allele	Obs.	Exp.	SR	Obs.	Exp.	SR
Low	*Rrn1-1*	38	38	0.0	3	3	−0.0
	Rrn1-2	12	12	−0.0	1	1	0.0
Medium	*Rrn1-1*	24	22.9	0.2	6	7.1	−0.4
	Rrn1-2	21	22.1	−0.2	8	6.9	0.4
High	*Rrn1-1*	25	21.7	0.7	5	8.3	−1.1
	Rrn1-2	22	25.3	−0.7	13	9.7	1.1

Standardized residual [SR = Obs. − Exp.)/(Exp.)$^{1/2}$] follow an asymptotically normal distribution with mean zero and unit variance; absolute values > 1.96, 2.58, and 3.29 are significant at $P = 0.05$, 0.01, and 0.001, respectively. Obs., observed number; Exp., expected number.

Table 5. Estimated standardized u values for associations in the model [*LH*] [*SH*]

		Humidity		
	Allele	Low	Medium	High
Rrn1	*1*	3.31*	−1.41	−2.31†
	2	−3.31*	1.41	2.31†
Rrn2	*1*	2.62‡	−1.35	−2.17†
	2	−2.62‡	1.35	2.17†

Standardized u values follow an approximately normal distribution with mean zero and unit variance based on the hypothesis of absence of such a term (see Table 4).
*P = 0.05; † P = 0.01; ‡ P = 0.001.

associations; however, it may not be appropriate to interpret them because they are nested within the third-order *LSA* association (9). At low, medium, and high elevations estimated standardized u values for the *LSA* association took absolute values 2.30, 0.50, and 2.07. At low altitudes the u values were positive for allelic combinations 11 (allele 112 with allele 107) and 22 but negative for allelic combinations 12 and 21, whereas at high altitudes the u values were negative for the 11 and 22 combinations but positive for the 12 and 21 combinations. Hence, at low elevations the 11 and 22 allelic combinations were in excess, whereas the reverse was true at high elevations.

Parallel analyses were carried out for *Rrn1* and *Rrn2* with the seven remaining environmental factors. Table 6 gives the best-fitting models and likelihood ratio statistics for each of the nine environmental variables. Saturated models give the best fit for five of the variables. This indicates that allelic state at the two rDNA loci depends not only on allelic state at the other locus but also on the environmental factors *A*, *I*, *O*, *U*, and *J*. The model [*LT*] [*ST*] indicates that both loci are associated with annual temperature, whereas the model [*SL*] [*SE*] indicates that the two loci are associated with each other, but only *Rrn1* is associated with evaporation. The model [*LS*] [*R*] suggests the two loci are associated with each other but both are independent of rainfall. Overall, the rDNA loci were associated with eight of the nine environmental variables. The models of Table 6 also indicate that the rDNA loci are associated in either pairwise or in three-way interactions with the environmental factors.

DISCUSSION

The results of the present study of genetic variability in different habitats reveal several features of the individual and joint effects on adaptedness of the 20 known alleles of rDNA loci *Rrn1* and *Rrn2*. Our analyses show that both allelic and genotypic diversity are substantial within and among populations, particularly populations of *H.S.* In total, 17 alleles and 36 genotypes were observed in our sample of *H.S.* The proportion of among- to within-population genetic variability was larger for genotypic (65%) than for allelic (53%) variability; thus, two-locus genotypic composition indicates that genetic differentiation among populations from different habitats is larger than indicated by the allelic composition of the populations. Correlations between genotypes and environmental conditions have often been interpreted as evidence for adaptation (e.g., ref. 10). Our analyses showed that allelic frequencies and two-locus genotypic frequencies often depended on complex interactions among alleles of *Rrn1* and *Rrn2* with each other and with eight among nine factors of the physical environment; thus, the adaptive properties of the rDNA alleles and genotypes differ from habitat to habitat. The results also show that, although most populations of *H.S.* were conspicuously polymorphic at the allelic and genotypic levels, one allele was predominant at each locus (allele 112 of *Rrn1* and allele 107 of *Rrn2*) and that one genotype (112,112, 107,107) was predominant in the great majority of populations (Table 3). The population behavior of alleles 112 and 107 in most habitats occupied by *H.S.* was therefore that of well-adapted wild-type alleles. Six other alleles (alleles 110, 109, 108, and 108a of *Rrn1* and alleles 106 and 105 of *Rrn2*) were locally frequent (f = 0.05–0.20) in ecologically unusual sites; these alleles form genotypes that, in polymorphic association with genotype 107,112 (Table 3), appear to enhance adaptedness in specialized habitats. Ten alleles were rare ($f < 0.01$) or absent in all of our populations of *H.S.*; the population behavior of these rare alleles in nature varied from that of subvitals to semilethals. The rare rDNA alleles have also been found to have adverse effects on survival under greenhouse conditions (3); these rare rDNA alleles are nearly always found in association with alleles 112 or 107, often in three-allele genotypes featuring a "compound" allele (Table 1), which suggests that the survival of rare rDNA alleles in populations depends in part on "hitchhiking" on favored wild-type alleles.

Similar individual and joint effects on the adaptedness of specific rDNA alleles and genotypes were no less evident in *H.V.* On a worldwide basis, *Rrn1* is nearly fixed for allele 112 (Table 2), whereas *Rrn2* is dominated by alleles 104 and 107 (f = 0.65 and 0.30, respectively). Allele 104 is predominant in some areas and allele 107 in other areas (especially areas with Mediterranean climates). All of the 17 remaining alleles were rare or absent in our samples of *H.V.* Overall, rDNA variability is much smaller in *H.V.* (only 8 alleles and 10 genotypes) than in *H.S.* (17 alleles and 36 genotypes) and allelic and genotypic frequencies are usually very different in the two subspecies. The most obvious cases are those of allele 104, which is infrequent in *H.S.* (f = 0.02) but predominant in the majority of populations of *H.V.*, and the moderately frequent alleles of *H.S.* (110, 109, 108, 108a, 106, and 105), which are absent or rare in *H.V.* The differences in the arrays of alleles and genotypes in the two subspecies are not surprising for two reasons; wild barley occupies a wider range of habitats than cultivated barley and conditions of life are very different under cultivation than in nature.

The effects of natural selection on rDNA alleles under conditions of cultivation are well illustrated by the results of a study (2) on an experimental population of *H.V.* which was grown without conscious selection in large plots (>15,000 reproducing adults per generation) for 53 generations. Initial frequencies of alleles 112, 104, and 107 were 0.89, 0.61, and 0.32, respectively, in this population (close to worldwide frequencies for *H.V.*). However, by generation 23 allele 112 had become fixed (f = 1.00) in *Rrn1*, and by generation 53 the frequency of allele 104 had decreased to 0.30, the frequency of allele 107 had increased to 0.70, and the other alleles of *Rrn2*, all originally present in low frequency, had disappeared. Thus, in the Mediterranean climate in which this experimental population was grown, allele 112 was unchallenged as the wild-type allele of *Rrn1*, alleles 104 and 107 were competitive as wild-type alleles of *Rrn2*, and the population

Table 6. Best-fitting models for two rDNA loci and nine environmental factors

Factor	Model	G^2	df	P
Humidity	[*LH*] [*SH*]	3.96	3	0.27
Evaporation	[*SL*] [*LE*]	6.03	4	0.20
Annual temperature	[*LT*] [*ST*]	6.60	3	0.09
Annual rainfall	[*LS*] [*R*]	8.70	6	0.19
Altitude	[*LSA*]	0	0	
Latitude	[*LSI*]	0	0	
Longitude	[*LSO*]	0	0	
Mean August temperature	[*LSU*]	0	0	
Mean January temperature	[*LSJ*]	0	0	

G^2, likelihood ratio statistic; df, degrees of freedom.

behavior of the infrequent and rare alleles of *H.V.* (Table 2) ranged, as in *H.S.*, from that of subvitals to semilethals. These results are consistent with those of other studies of rare alleles (e.g., ref. 1).

There is, in addition to the variability associated with the differing numbers of subrepeats of the IGS region, another type of variability in the rDNA of barley: continuous variation in copy number. A study of copy number variations in 10 populations of *H.S.* from Israel (11) suggests that adaptedness is much less closely correlated with the quantity of rDNA, as measured by numbers of copies and numbers of subrepeats in the IGS region, than with probable qualitative (nucleotide sequence) differences in the rDNA of the specific alleles marked by the slvs.

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