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Effects on adaptedness of variations in ribosomal DNA copy number in populations of wild barley (*Hordeum vulgare* ssp. *spontaneum*)

(multiplicity of ribosomal DNA/Mendelian ribosomal DNA loci/ribosomal DNA alleles)

QIFA ZHANG*, M. A. SAGHAI MAROOF†, AND R. W. ALLARD‡

Department of Genetics, University of California, Davis, CA 95616

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ABSTRACT **Twenty alleles, 12 at Mendelian locus *Rrn1* and 8 at locus *Rrn2*, control rRNA genes [ribosomal DNA (rDNA)] variability in barley. These alleles differ strikingly in their effects on adaptedness. In the present study, we determined variation in the copy number of 101 accessions of wild barley plants from 10 ecologically diverse sites in Israel and examined relationships between rDNA copy number and adaptedness. The average multiplicity of rDNA per haploid genome was 1881 copies and the average numbers of copies for *Rrn1* and *Rrn2* were 962 and 917, respectively. The total number of copies as well as the number of copies for *Rrn1* and *Rrn2* varied widely from plant to plant within sites and also from site to site. The predominant allele of *Rrn2* had somewhat more copies on the average than the other alleles of this locus but differences between the predominant allele and other alleles of *Rrn1* were not statistically significant. Overall, the results indicated that differing amounts of rDNA resulting from variations in copy number and/or number of subrepeats in the intergenic spacer region were not closely associated with adaptedness. This suggests that the high adaptedness of a few specific alleles results in large part from adaptatively favorable nucleotide sequences in the transcription units and/or the intergenic spacer regions of the favored alleles—i.e., that adaptedness in barley depends on the quality more than on the quantity of rDNA present.**

rRNA genes [ribosomal DNA (rDNA)] are repeated sequences of DNA arranged in tandem arrays at one or more chromosome locations. Each array consists of a transcriptional unit that codes for the 18S, 5.8S, and 26S complex of cytoplasmic rRNA and an intergenic spacer (IGS) region that separates adjacent transcription units (for reviews, see refs. 1 and 2). The IGS region includes an array of repeated sequences referred to as subrepeats. The number of subrepeats is usually variable within species leading to disjunct variations in the length of the IGS region that are detectable by Southern blot hybridization. The rDNA arrays of individual plants may also differ with respect to nucleotide sequences and/or base modifications in the transcription units and/or the IGS region (for review, see ref. 2). A fourth mode of rDNA variation has also often been observed, namely variation in copy number of rDNA per haploid genome (e.g., refs. 3–8). However, the copy number is a quantitative character that varies in a continuous series from individual to individual; copy number is difficult to quantify and it has rarely been measured precisely.

In cultivated barley *Hordeum vulgare* ssp. *vulgare* (*H.V.*) and in its conspecific wild ancestor *H. vulgare* ssp. *spontaneum* (*H.S.*), two Mendelian loci, *Rrn1* and *Rrn2*, associated with the nucleolar organizer regions of chromosomes 6 and 7, respectively, control rDNA variability (9, 10). Twenty spacer-length variants (slvs) have been identified in the IGS region. The 20 slvs are organized in two families, one comprised of a regularly complete 8-step ladder (slvs 100–107) 4625–5430 base pairs (bp) long in the nucleolar organizer region of chromosome 7 and the other a 12-step ladder (slvs 108a–118) 5545–6695 bp long in the nucleolar organizer region of chromosome 6. All slvs differ from their immediately adjacent neighbors in the ladders by a 115 bp subrepeat except slv 108a (the shortest variant in *Rrn1*), which is ≈42 bp shorter than slv 108 and ≈73 bp longer than slv 107. The 8 shorter slvs (100–107) segregate and serve as markers of the eight rDNA alleles of Mendelian locus *Rrn2* whereas the 12 longer slvs segregate and serve as markers of alleles 108a–118 of *Rrn1*. Thus, in barley, an rDNA allele is comprised of large numbers of tandem repeats of a transcriptional unit plus an associated IGS region that is variable in length.

There have been two studies of the effects of natural selection on the rDNA alleles marked by the 20 slvs of barley. One study (9) was of composite cross II, an experimental population of *H.V.* that, after its synthesis in 1929 from intercrosses among 28 barley varieties representing all of the major barley-growing areas of the world, has been grown annually in large plots (>15,000 reproducing adults) without conscious selection in the Mediterranean climate of Davis, CA. Allele 112 of *Rrn1* and alleles 104 and 107 of *Rrn2* were present in frequencies 0.89, 0.62, and 0.32, respectively, in the initial generation of composite cross II; these frequencies are very close to worldwide frequencies of these alleles in *H.V.* Steady directional changes occurred in the frequencies of alleles 112, 104, and 107 over generations and by generation 53 their frequencies had become 1.00, 0.30, and 0.70, respectively; these allelic frequencies are very close to estimates obtained from assays of large samples of *H.V.* from Mediterranean climates. All other alleles originally present disappeared rapidly from composite cross II. These changes in allelic frequencies indicate that rDNA allele 112 was unchallenged as the "wild-type" allele of *Rrn1*, that allele 107 was slightly favored on the average over allele 104 at locus *Rrn2*, and that the population behavior of the other 17 alleles was that of subvitals or semilethals in the Mediterranean climatic conditions in which composite cross II was grown. The other study (11) established that the frequencies of the rDNA alleles and genotypes varied widely from habitat to habitat in 18 populations of *H.S.* from ecologically diverse

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Abbreviations: slv, spacer-length variant; *H.S.*, *Hordeum vulgare* spp. *spontaneum*; *H.V.*, *H. vulgare*, spp. *vulgare*; IGS region, intergenic spacer region; rDNA, rRNA genes or ribosomal DNA.

*Present address: Department of Agronomy, Huazhong Agricultural University, Wuhan, People's Republic of China.
†Department of Crop and Soil Environmental Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.
‡Department of Agronomy and Range Science, University of California, Davis, CA 95616.

habitats in Israel and Iran. In the majority of these populations, alleles 107 (*Rrn2*) and 112 (*Rrn1*) behaved as predominant and widely adapted wild-type alleles. Alleles 110, 109, 108, and 108a of *Rrn1* and alleles 106 and 105 of *Rrn2* were moderately frequent in specialized habitats whereas the 12 remaining alleles were infrequent or rare in all populations. Discrete multivariate log–linear analyses revealed that allelic and genotypic frequencies were correlated with eight among nine factors of the physical environment and that two-locus genotypic frequencies were often associated with specific factors of the environment. These two studies thus indicate that natural selection acting on the rDNA alleles of *Rrn1* and *Rrn2* plays a major role in the development and the maintenance of the observed patterns of molecular and genetic organization of rDNA variability in *H.V.* and *H.S.* (for review, see ref. 12).

In the present report, we focus on quantitative variability in the multiplicity of rDNA copy number in populations of *H.S.* from ecologically diverse habitats in Israel. We report (*i*) the range in copy number and the mean copy number per haploid genome in the 101 plants of our sample, (*ii*) variations in the copy numbers of plants from different habitats, (*iii*) copy numbers of rDNA loci *Rrn1* and *Rrn2*, and (*iv*) variations in copy numbers of various alleles of *Rrn1* and *Rrn2*. Our results indicate that the differing amounts of rDNA associated with differences in copy number and/or with length differences in the IGS regions are not closely associated with adaptedness, which suggests that the high adaptedness of a few specific alleles stems in large part from favorable nucleotide sequences in the transcription units or the IGS regions of these alleles.

MATERIALS AND METHODS

Genetic Materials. The genetic materials of this study were 101 accessions of *H.S.* from Israel obtained from the U.S. Department of Agriculture World Barley Collection. These accessions were from 10 collection sites representing environmentally diverse habitats in Israel (11, 13). One plant from each accession was assayed for rDNA allelic composition and for rDNA copy number.

DNA Extraction and Purification. Cellular DNA was extracted from leaf tissue as described (9). The DNA samples were centrifuged twice to equilibrium in CsCl and ethidium bromide gradients in a TV865 rotor (Sorvall) at 58,000 rpm, followed by isopropanol extraction. The DNA recovered was dialyzed extensively in a buffer containing 10 mM NH_4Ac and 0.1 mM EDTA. To rid the samples of trace amounts of RNA, which may cause serious errors in estimating copy numbers, the DNA was digested with DNase-free RNase (15 mg/ml) for 3 hr at 37°C, extracted with phenol, phenol/chloroform, and chloroform, and ethanol-precipitated in a microcentrifuge, and the pellet was resuspended in DNA storage buffer (0.1 mM EDTA/10 mM NH_4Ac). The ratios of adsorbance at two wavelengths (A_{260}/A_{280}) ranged between 1.80 and 1.90 for the 101 samples. Precautions were taken to avoid inaccuracies in DNA quantification; we found that the following two-step process produced highly repeatable estimates. Each DNA sample was diluted 1:40 and its A_{260} was read in a spectrophotometer. By using this estimate as a reference, a 5-μg portion of DNA per sample was removed from the tube and diluted to 1 ml, and the A_{260} was again measured. The 1 ml of DNA solution was saved in a microcentrifuge tube and used for copy-number analysis. Proofreadings using the entire contents of tubes showed that variations were within 1% of the total DNA concentration.

Measuring Total DNA Copy Number of Individual Plants. The slot-blot technique (14) was used to assay the copy number of rDNA for each sample. A piece of Biodyne membrane was boiled for 5 min in distilled water and equilibrated with 1 M NH_4Ac. Three layers of Whatman 3MM paper (preequilibrated with 1 M NH_4Ac) were placed beneath the membrane and assembled into a slot-blot apparatus (Schleicher & Schuell). Exactly 200 ng (about 40 μl) of barley DNA was pipetted into a microcentrifuge tube containing 230 μl of DNA storage buffer and 30 μl of 1.0 M NaOH. The tubes were then boiled for exactly 10 min and cooled on ice. Immediately before loading, 90 μl of 5 M NH_4Ac was added to each tube, and the mixture was vortex-mixed (homogenized). The homogenate was then loaded onto the membrane, each slot was rinsed with 200 μl of 1 M NH_4Ac, and excess liquid was removed by vacuum. The blot was then air-dried for several hours, baked at 80°C for 2 hr, and hybridized to an [α-^{32}P]dCTP-labeled pTA71 plasmid that contains the entire 9-kilobase wheat rRNA gene repeating unit (15). After brief air-drying, the blot was placed on preflashed x-ray film. The resulting autoradiographs were scanned under a densitometer to determine the relative density of each slot; relative density is proportional to the amount of rDNA in the sample.

To reduce errors from loading and from nonuniform hybridization, only the middle 24 of 96 slots of the blotter were loaded. Each sample was replicated three times within each blot, following a completely randomized design. Three concentration gradients (0.30 ng, 0.60 ng, and 0.90 ng) of the plasmid pTA71, each loaded with 200 ng of carrier DNA and replicated twice in a blot, were used as internal standards: this allowed comparisons of the amounts of hybridization signal for samples in different blots and also checks of the linearity of responses obtained from individual blots. To obtain estimates of between-blot experimental error, many blots were duplicated and many of the DNA samples were tested in different blots.

The proportion of rDNA (P_r) in a sample was estimated using the conversion (slightly modified), as described (16),

$$P_r = (D_T \cdot S_v \cdot Q_I \cdot S_T)/(D_v \cdot S_I^2 \cdot Q_T). \quad [1]$$

In Eq. 1, D_T and D_v are, respectively, the densitometer readings of the plant DNA sample and plasmid standard, S_I and S_v are the sizes (bp) of the insert and the vector plus the insert, Q_I and Q_T are the amounts (ng) of the insert and the plant DNA sample, and S_T is the average length (bp) of the rDNA sequence in the plant DNA sample. The estimation of S_T will be described below.

The absolute copy number (CP) of the rDNA sequence per haploid genome can be calculated as

$$CP = C \cdot P_r / S_T, \quad [2]$$

in which C, the size of the haploid genome, is 5.5×10^9 bp in barley (16). Substituting Eq. 1 into Eq. 2 gives the operational equation for calculating copy number

$$CP = (C \cdot D_T \cdot S_v \cdot Q_I)/(D_v \cdot S_I^2 \cdot Q_T). \quad [3]$$

Estimating Copy Number of *Rrn1* and *Rrn2*. In determining the average length and copy number of rDNA *Rrn1* and *Rrn2*, the total DNA of each sample was digested to completion with *Sst* I and then fractionated in an agarose gel. The DNA was immobilized on a Biodyne membrane, which was then baked and hybridized with a radioactively labeled probe. The resulting autoradiographs were scanned to determine the relative intensity of the restriction fragment at each locus and thus the relative amount of DNA associated with the rDNA variant(s) at each locus. The average length of the rDNA fragment(s) of *Rrn2* (S_2) can be estimated by

$$S_2 = 3880 + \sum_i^8 l_i \omega_i, \quad [4]$$

and that of *Rrn1* (S_1) can be estimated by

$$S_1 = 3800 + \sum_{i}^{12} l_i\omega_i. \quad [5]$$

In Eqs. **4** and **5**, 3880 is the size (bp) of the invariant fragment containing a major portion of the transcription unit (9), l_i is the length of ith variant fragment made up mostly of the IGS region, ω_i is the proportional density of the ith fragment summed over all of the fragments of *Rrn1* or *Rrn2*, and 8 and 12 are the total number of alleles at *Rrn1* and *Rrn2*, respectively. The density readings of the variants present at each locus, therefore, provide a means of estimating the proportion of rDNA at each locus. As an example, for an individual plant that was homozygous for slvs 107 (*Rrn2*) and 112 (*Rrn1*), density readings were 73 for slv 107 (which lies in a 5430-bp *Sst* I fragment) and 160 for slv 112 (which lies in a 6005-bp IGS fragment); thus 68.7% and 31.3% of the total rDNA of an individual were associated with *Rrn1* and *Rrn2*, respectively. In an individual that had three slvs (104, 107, and 108, which lie in *Sst* I fragments 5085, 5430, and 5545 bp long), density readings were 58, 159, and 31, respectively; thus slvs 104, 107, and 108 made up 23.4%, 64.1%, and 12.5% of the rDNA, respectively, and 87.5% and 12.5% of the rDNA of the plant were associated with *Rrn2* and *Rrn1*, respectively. The absolute copy number of rDNA at the kth locus (CP_k) is estimated by

$$CP_k = C{\cdot}P_rP_k/S_k, \quad [6]$$

in which P_k is the proportion and S_k is the average size (bp) of the rDNA at that locus.

RESULTS

Variation in Copy Number per Haploid Genome. The number of copies of rDNA repeats per haploid genome, estimated using Eq. **3**, varied from a low of 792 to a high of 5048 for the 101 plants of our sample. The modal and the mean numbers of copies were 1500 and 1898, respectively. The distribution of the number of copies per plant is given in Fig. 1 from which it can be seen that the distribution curve is skewed toward the low side and that the copy number for the great majority of plants fell in the lower middle one-third of the range (800–2250 copies).

Estimates of copy numbers for about one-half of the 101 plants were based on three observations (replicates) from a single blot, whereas the estimates for the other half of the plants were based on five or more observations, usually from two but sometimes three blots. There were consequently two sources of within-plant error, a component due to variation within blots (348 df) and a component due to differences between blots (77 df). An analysis of variance on repeated observations of the same samples on different blots showed that differences due to these two sources of variation were not statistically significant (the ratio of mean square values was 1.28, $P > 0.07$). Consequently, the within- and between-blots components were pooled and the resulting mean square value (425 df) was used to estimate the within-plant mean square value for testing the significance of the between-plant component. The mean square value (100 df) due to differences among the 101 plants was nearly 40 times as large as the within-plant mean square value ($F = 39.4$ and $P << 0.001$). The least significant difference statistic, which was 460, was used to assess the significance of differences in copy numbers of individual plants; differences between plants greater than 460 copies are consequently significant at $P = 0.05$. Thus, although the copy numbers of the 101 individuals are distributed more or less continuously (Fig. 1), most of the individuals in our sample differed significantly from one another in copy number.

Variation in Copy Number from Site to Site. Statistics calculated from the original data indicated that variances of copy numbers from different sites were heterogeneous. A logarithmic transformation removed this heterogeneity in part but did not affect statistical significance; consequently, we used the original untransformed data in analyzing location effects on copy number. The between-site mean square value (9 df) was 2.36 times larger than the within-site mean square value (91 df), which is significant at $P = 0.01$. The least significant difference statistic, which was 689 copies, was used to test the significance of differences between sites (site 10 was excluded from these comparisons because it was represented by only one individual). Comparisons among sites (Fig. 2) show that between-site copy number differences were substantial and often significant. As examples, site 9, which was not significantly different from sites 5, 6, 7, or 8, was significantly different ($P < 0.05$) from all other sites; also sites 7 and 8 were significantly different from sites 1 and 2.

Copy Numbers of Alleles of *Rrn1* and *Rrn2*. Extensive surveys of IGS variability in barley (9–11) have shown that

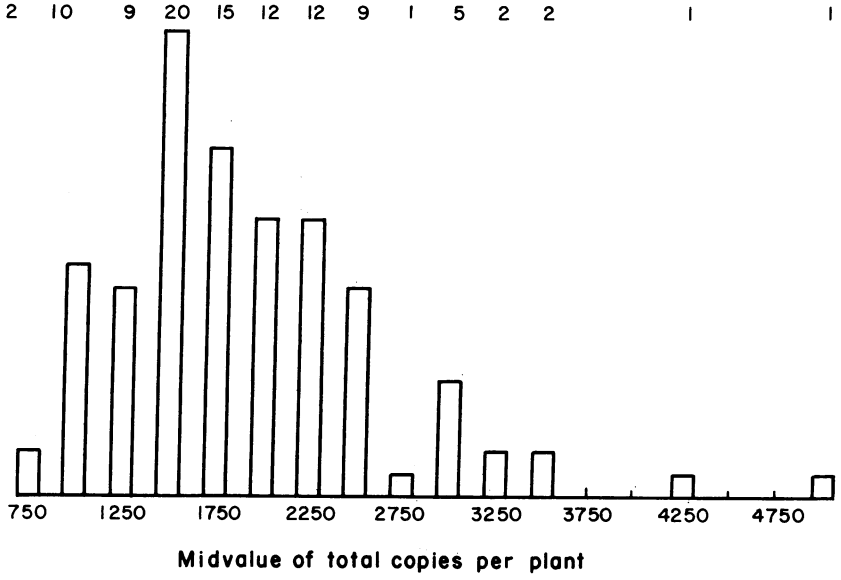

FIG. 1. Distribution of total copy numbers of rDNA for 101 accessions of *H.S.*

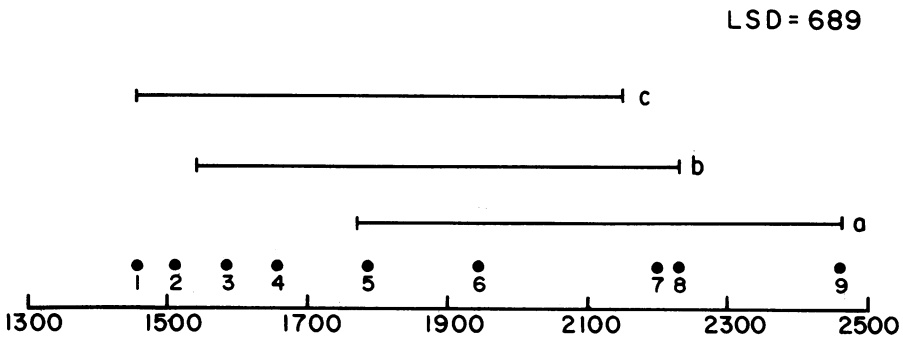

FIG. 2. Comparison of total copy number by the least significant difference (LSD) statistic for accessions of *H.S.* from nine locations, indicated by dots. Locations (dots) covered by the same line (a, b, or c) are not significantly different in copy number.

the great majority of individual barley plants (≈90%) have two slvs, including one from *Rrn1* and one from *Rrn2*. However, exceptional plants are found in barley populations that deviate from the above pattern in one of the following ways. (*i*) Both slvs are from *Rrn1* or *Rrn2*. (*ii*) Only one slv is present (always either slv 107 or slv 112) and it is present in both loci. (*iii*) Three slvs are present; some plants with three slvs are homozygous at one rDNA locus but heterozygous at the other rDNA locus whereas other plants with three slvs are homozygous for a compound allele (marked by two slvs at one locus) and also homozygous for a third allele at the other locus. (*iv*) Four slvs are present and the plant is heterozygous at both *Rrn1* and *Rrn2*. Four exceptional plants in our sample of 101 plants fell into one or another of these classes. Although there was no indication that the atypical genotypes of these plants affected copy number of any of the alleles involved, these four plants were excluded from analyses of copy number of alleles of *Rrn1* and *Rrn2*. Copy number for each locus was calculated for the remaining 97 plants by using Eq. **6**. Errors arising in the fractionating of the slvs from the two loci (e.g., losing DNA in electrophoresis on nonuniform blotting) were additional potential sources of variability. To assess the extent of potential experimental errors, DNA samples were extracted from two separate harvests from the same plants made at intervals of ≈2 months. The rank correlation of the relative proportions of rDNA from the same loci was 0.99 between the two harvests; thus the readings obtained were highly repeatable.

Copy numbers averaged over the 97 individuals assayed were 962 ± 444 for *Rrn1* and 917 ± 542 for *Rrn2*. Average copy numbers for the two loci, therefore, did not differ significantly. However, the large standard errors for copy numbers for both loci show that variations in rDNA multiplicity are very large for both loci. This is illustrated in Table 1 from which it can be seen that the amounts of rDNA at the two loci are very similar for some individuals (e.g., plants 4, 8, and 10) but very different for other individuals (e.g., copy number is higher for *Rrn1* than *Rrn2* in plants 7 and 9 but the opposite is true in plants 3 and 5).

Copy Numbers of the Individual rDNA Alleles. In previous studies a single allele, 112 of *Rrn1* and 107 of *Rrn2*, has been found to be predominant at each locus in *H.S.* (9–11). In the present study, 54 of the 97 individuals (0.56) were homozygous for allele 112 of *Rrn1* and 76 (0.81) were homozygous for allele 107 of *Rrn2*. Other alleles present were alleles 108 (0.26), 109 (0.10), 108a (0.03), 110 (0.02), and 113 and 111 (<0.01) of *Rrn1* and alleles 106 (0.17) and 103 and 104 (<0.02) of *Rrn2*. Copy numbers (mean ± SEM) were 1000 ± 480 for allele 112 and 817 ± 600 for the other alleles of *Rrn1* and 1034 ± 444 for allele 107 and 702 ± 340 for the other alleles of *Rrn2*. The range in copy numbers was, therefore, very high for all alleles and the differences in copy numbers between different alleles were not statistically significant, except that the difference between allele 107 and all other alleles of *Rrn2*, which was significant at $P = 0.001$.

DISCUSSION

We have assayed copy number of rDNA per haploid genome and the number of copies associated with loci *Rrn1* and *Rrn2*. The mean number of copies for 97 plants whose slvs (alleles) could be assigned unambiguously to the two loci was 1881 per haploid genome, and the means were 962 for *Rrn1* and 917 for *Rrn2* (sum = 1879). The two estimators are thus consistent in estimating total copies. It is clear from Eq. **3** that the estimation of the total number of copies is independent of the size of the target sequence when the sizes of the plasmid and insert are known; consequently, sequence-length information is not necessary in estimating total copy number. However, both the average length of the sequence and the average

Table 1. Densitometer readings for relative proportions of rDNA at the two loci in DNA samples extracted from plant tissues of two harvests of the same individuals

	Harvest 1			Harvest 2		
Plant	*Rrn1*	*Rrn2*	*Rrn2/Rrn1*	*Rrn1*	*Rrn2*	*Rrn2/Rrn1*
1	20	30	1.50	20	34	1.70
2	35	62	1.78	42	66	1.56
3	13	36	2.70	28	68	2.44
4	274	316	1.13	142	181	1.27
5	89	240	2.70	100	215	2.12
6	48	70	1.44	91	144	1.56
7	131	32	0.25	91	45	0.48
8	31	38	1.22	44	56	1.27
9	160	19	0.14	88	14	0.16
10	55	50	0.92	30	30	1.0

Rank correlation (harvest 1 with harvest 2) = 0.99. Absolute values for different plants in this table cannot be compared directly because the total amounts of DNA in each slot were not precisely equilibrated and the readings were obtained from different films.

length of the allele present at a particular locus are required to estimate copy numbers associated with individual loci. Without information of sequence-size differences, the multiplicity of the locus containing the shorter DNA sequences would be underestimated and that of the locus with the longer fragments would be overestimated. Our values may represent slight underestimates because the homology between the standard and the probe (wheat with wheat) is complete but the homology between barley rDNA and the wheat probe may be less than complete. This, however, should not affect interpretations of the extent of copy number polymorphism in this species. We assumed in estimating rDNA copy numbers of individual plants that the genomic size (*C* value) is constant from one plant to another in the entire species. However, there is evidence suggesting that intraspecific variation in nuclear DNA content exists in some plant species (17) and, if this is true in *H.S.*, our estimates of the multiplicity represent indices of relative proportions of rDNA in the genome rather than absolute copy numbers of individual plants. This would not, however, affect conclusions regarding the relative multiplicity of rDNA in *H.S.*

Drastic changes in rDNA copy number during different stages of the life cycle within the same plant have been reported in some studies. In the present study the relative amounts of rDNA at *Rrn1* and *Rrn2* were almost identical from DNA samples extracted from harvests made 2 months apart. This indicates, at the least, that the relative proportions of rDNA did not change at the two loci from the first to the second harvest.

A main feature of our results was the wide range of variation in rDNA copy numbers in individual plants of *H.S.* Similar large differences in rDNA copy number have been reported in other plant species (4–8). In this connection we note that two estimates in *H.V.* (18, 19), although different from each other, fall within the range of copy number variation observed in the present study; hence, the difference between these two estimates may be due to the specific stocks that were assayed. A direct implication of large amounts of within-species variation in copy number is that hybridization between individuals of low and high copy numbers may lead to imprecise pairing between chromosome segments during meiosis and that this might in turn lead to unequal crossing over that generates new copy number variants. Thus, copy-number variants, once present, might have the potential of increasing the extent of variation through the recombination process. It is, therefore, not surprising to discover extensive copy-number variation in repetitive gene families.

Our data suggest that there are significant differences in the total copy number among plants from different locations and, hence, that copy-number variation may be related to environmental factors. As an example, individuals collected from locations with exceptionally high or low rainfall tended to have the lowest copy numbers. However, even though the number of locations and number of individuals per location were small, the erratic variations observed suggest that copy number is, at the most, weakly associated with adaptedness.

The frequencies of the 20 rDNA slvs (alleles) that have been identified in *H.V.* and *H.S.* are strongly correlated with environment, indicating these alleles are affected by natural selection (9–11). In *H.S.*, slv 112 (6005 bp) is predominant in *Rrn1* and slv 107 (5430 bp) is predominant in *Rrn2* (9–11); in *H.V.*, slv 104 (5085 bp) is more frequent than slv 107 in many environments (11). In *H.S.*, 6 other alleles (110, 109, 108, and 108a of *Rrn1* and 106 and 105 of *Rrn2*) appear to confer superior adaptedness in specialized habitats. The remaining 11 alleles (118, 117, 116, 115, 114, 113, 111, 103, 102, 101, 100), which vary from 6995 to 4625 bp long, are infrequent or rare in all populations of *H.V.* and *H.S.* and they have also been found to have adverse effects on survival under greenhouse conditions (10). These wide differences in the ecological preferences and frequencies of the 20 rDNA alleles marked by the slvs are therefore noteworthy, especially because the only other differences among the alleles thus far identified are the number of apparently identical 115-bp subrepeats in the IGS region. One possible explanation for the differing adaptive properties of the alleles marked by the slvs is that adaptedness stems solely or largely from the differing numbers of subrepeats in the IGS region. In *Xenopus* there is experimental evidence suggesting that the IGS region has the property of enhancing transcription rates and that the transcription rates of the longer spacers may be higher than those of the shorter spacers (20). It is not readily apparent, however, how differences of one or a few subrepeats lead to very large differences in adaptedness; e.g., why is *H.V.* allele 104, with one to four more subrepeats than alleles 100, 101, 102, or 103 and one or two fewer subrepeats than alleles 105 and 106, by far the most successful among these 7 alleles of *Rrn2* (11). Similarly, it is difficult to see why allele 112 of *Rrn1*, which has one or two more subrepeats than alleles 110 and 111 and one to six fewer subrepeats than alleles 113–118, is much better adapted in both *H.S.* and *H.V.* than its neighbors on the ladder. One alternative explanation is that the 115-bp subrepeats, although identical in length, may differ in sequence and that slvs 104, 107, and 112 carry sequences that are highly favored in selection. Another possible explanation is that the transcription units of rDNA alleles such as 104, 107, and 112 may carry sequences that are especially favored by selection. It is clear from the data of the present experiment that differences in amounts of rDNA, whether stemming from copy-number variations or from numbers of subrepeats in the IGS region, are not closely related to adaptedness. We consequently favor the hypothesis that adaptedness is more a function of the quality than of the quantity of rDNA in barley. Clues to the resolution of this issue might be obtained by sequencing the slvs and transcription units of a selected set of the rDNA alleles.

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