Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 8014–8018, December 1984
Population Biology

Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics

(ribosomal DNA spacer-length variation/restriction fragment-length polymorphisms/*Rrn1*/*Rrn2*)

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Contributed by R. W. Allard, August 27, 1984

ABSTRACT **Spacer-length (sl) variation in ribosomal RNA gene clusters (rDNA) was surveyed in 502 individual barley plants, including samples from 50 accessions of cultivated barley, 25 accessions of its wild ancestor, and five generations of composite cross II (CCII), an experimental population of barley. In total, 17 rDNA sl phenotypes, made up of 15 different rDNA sl variants, were observed. The 15 rDNA sl variants comprise a complete ladder in which each variant differs in length from adjacent variants by ≈115 nucleotide pairs. Studies of four rDNA sl variants in an F_2 population showed that these variants are located at two unlinked loci, *Rrn1* and *Rrn2*, each with two codominant alleles. Using wheat–barley addition lines, we determined that *Rrn1* and *Rrn2* are located on chromosomes 6 and 7, respectively. The nonrandom distribution of sl variants between loci suggests that genetic exchange occurs much less frequently between than within the two loci, which demonstrates that *Rrn1* and *Rrn2* are useful as new genetic markers. Frequencies of rDNA sl phenotypes and variants were monitored over 54 generations in CCII. A phenotype that was originally infrequent in CCII ultimately became predominant, whereas the originally most frequent phenotype decreased drastically in frequency, and all other phenotypes originally present disappeared from the population. We conclude that the sl variants and/or associated loci are under selection in CCII.**

Eukaryotic ribosomal RNA genes (known as ribosomal DNA or rDNA) are organized as families of tandemly repeated genes some or all of which may comprise the nucleolar organizer regions of chromosomes (for a review, see ref. 1). Ribosomal RNA (rRNA) is synthesized as a single precursor RNA, subsequently processed into the mature 17S, 5.8S and 25S rRNAs. Each repeat unit of rDNA contains a single rRNA transcription unit as well as an intergenic spacer (IGS) region that separates the transcription units of adjacent repeat units (Fig. 1). The IGS region of each repeat unit contains an array of tandemly repeated sequences, referred to as "subrepeats," which are typically 100–300 base pairs (bp) in length in plants.

Within most species the length of the subrepeat varies by no more than a few base pairs, while the number of tandem copies of subrepeats within rDNA repeats is extremely variable. The result of variation in the number of tandem copies of subrepeats is variation in the length of the IGS region; this variation can be detected by restriction enzyme and Southern blot analysis of rRNA (e.g., refs. 2–7). Genetic analyses of rDNA spacer-length (sl) variants indicate that these variants tend to be distributed nonrandomly among arrays with respect to chromosomal location (2–5), with a single known exception (6). This relative homogeneity is attributable to molecular mechanisms such as unequal exchange and gene conversion.

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Little is known about the extent of genetic variability in and the genetic behavior of rDNA repeats. In this paper we report (*i*) the extent of rDNA sl variation and levels of polymorphism for rDNA sl phenotypes and rDNA sl variants in cultivated barley (*Hordeum vulgare* L.) and in its wild ancestor (*Hordeum spontaneum* L.); (*ii*) the Mendelian inheritance of four rDNA sl variants located at two loci; (*iii*) the degree of homogeneity of rDNA arrays; (*iv*) the population dynamics of sl variants and sl phenotypes over 54 generations in composite cross II (CCII); and (*v*) confirmation by addition-line analysis of the chromosomal locations of the two rDNA-containing regions in barley.

MATERIALS AND METHODS

Genetic Materials. The materials surveyed for rDNA sl variation included samples from 50 accessions of cultivated barley, from 25 accessions of its wild ancestor, and from 71 to 79 families from generations F_8, F_{13}, F_{23}, F_{45}, and F_{53} of CCII. The Mendelian inheritance of rDNA sl variants was studied in two F_2 populations. Wheat–barley addition lines were used to locate rDNA loci on the barley chromosomes.

DNA Preparation. Total cellular DNA was isolated from individual seedlings by a modification of the method of Murray and Thompson (8). Freeze-dried tissue (0.75 g, dry weight) was powdered with a mechanical mill, dispersed in 15 ml of extraction buffer (50 mM Tris, pH 8.0/0.7 M NaCl/10 mM EDTA/1% hexadecyltrimethylammonium bromide/0.1% 2-mercaptoethanol), and incubated at 60°C for 30–60 min with occasional mixing by gentle swirling. Chloroform/octanol, 24:1 (vol/vol) (10 ml), was added, and the solution was mixed by inversion to form an emulsion that was centrifuged at 5125 × g (IEC Clinical) for 10 min at room temperature. The aqueous phase was removed, and 2/3 vol of isopropanol was added and mixed by two-to-four quick, gentle inversions. The precipitated DNA was lifted out with a glass hook, transferred to a tube containing 20 ml of 76% ethanol/10 mM NH_4OAc for 20 min, and then dissolved in 1.5 ml of 10 mM NH_4OAc/0.25 mM EDTA. This method yields ≈200 μg of DNA per 0.75 g of dry tissue.

Detection of rDNA sl Variants. One microgram of DNA was digested to completion with two units of *Sst* I for 16 hr at 37°C. Electrophoresis was in 1.1% agarose and 100 mM Tris acetate/12.5 mM Na acetate/1 mM EDTA, pH 8.1 at 2 V/cm for 36 hr. Under these conditions, 5- to 6-kb fragments move 11–14 cm from the origin, and barley rDNA sl variants can be resolved unambiguously. DNA was transferred from these gels to nitrocellulose filter paper as described by Southern (9). The filters were hybridized to ^{32}P-labeled pTA71, a clone of a wheat rDNA repeat (10). Nick translation and hybridization were done essentially as described by Rigby *et al.* (11) and Maniatis (12).

Abbreviations: rDNA, ribosomal DNA; sl, spacer length; CCII, composite cross II; IGS, intergenic spacer region; bp, base pair(s).

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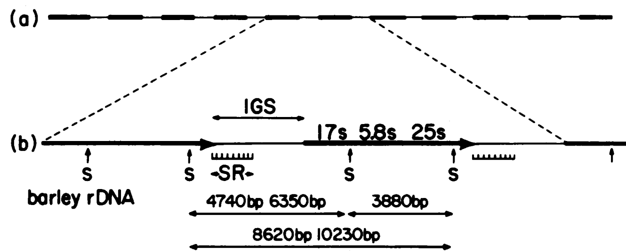

FIG. 1. Ribosomal RNA gene organization in barley. Line a represents the tandem array of ribosomal RNA (rRNA) genes that comprise each nucleolus organizer. In barley hundreds of tandem repeats lie at each nucleolus organizer. rRNA transcription units are represented by heavy horizontal lines and intergenic spacer (IGS) regions by light lines. Line b expands two complete repeat units and illustrates the tandem subrepeats in each IGS. "S" and the vertical arrows indicate the *Sst* I cleavage sites. Variation in the number of subrepeats (SR) results in variation in the length of the *Sst* I fragment carrying the IGS.

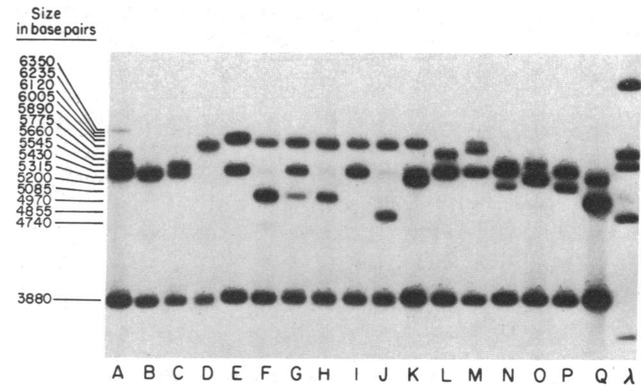

FIG. 2. Autoradiograph of Southern blot showing 17 (A–Q) different rDNA sl phenotypes observed in a survey of *H. vulgare* and *H. spontaneum*. The right-most lane shows phage λ *Eco*RI marker fragments.

Enzyme Electrophoresis. Enzyme electrophoresis was carried out by following the procedures of Kahler and Allard (13).

RESULTS AND DISCUSSION

Survey of rDNA sl Variation in *H. vulgare* and *H. spontaneum*. The following materials were assayed in our survey of rDNA sl variation in cultivated barley and its wild ancestor: (*i*) 2 individuals in each of 50 barley varieties (100 total individual plants) representing the major barley-growing regions of the world (28 of these 50 varieties were parents of CCII); (*ii*) 1 individual from each of 25 accessions of *H. spontaneum* (17, 6, 1, and 1 from Israel, Syria, Iran, and Afghanistan, respectively) and; (*iii*) 1 individual from each of 79, 75, 73, 71, and 79 families derived from reserve seed stocks of generations F_8, F_{13}, F_{23}, F_{45}, and F_{53}, respectively, of CCII (each family was descended from a single randomly chosen seed). Thus, in total, 502 individual plants were assayed in this survey.

Restriction enzyme *Sst* I was chosen for rDNA sl analysis because it cleaves each of the several thousand barley rDNA repeat units twice, once on each side of the IGS as illustrated in Fig. 1. Thus, *Sst* I cleavage yielded two fragments of DNA from each rDNA repeat unit. In barley one *Sst* I fragment was invariant (≈3880 bp), whereas the other varied in length from 4740 to 6350 bp, presumably as a result of variation in the number of tandem subrepeats contained in the IGS region as argued below. The array of length-variable bands are termed here "rDNA sl variants." Thirteen distinct rDNA sl variants were found (Fig. 2 and Table 1); rDNA sl variants 9 and 14 were not observed in this survey but they have been seen in other *H. spontaneum* accessions that we assayed subsequently. Each of the 15 variants that were observed in barley differed from the adjacent variant by ≈115 bp; thus, the series forms a complete ladder. We conclude that subrepeats are 115 bp in length and that the 15 variants are the result of variation in the number of subrepeats. The shortest rDNA sl variant, designated rDNA sl variant 1, slv-1 (Fig. 2), lies in a 4740-bp *Sst* I fragment. The longest rDNA sl variant, designated slv-15, lies in a 6350-bp *Sst* I fragment.

Each individual barley plant was scored for the presence of specific rDNA sl variants. Only one sl variant was observed in plants with phenotypes B and D, whereas two sl variants were observed in eight phenotypes (C, E, H, I, J, L, P, and Q), three in five phenotypes (F, G, M, N, and O) and four in two phenotypes (A and K). Thus, in all, 17 distinct rDNA sl phenotypes were found (phenotypes A–Q). Phenotypes H and I were the most frequent in our sample of *H. vulgare*, whereas phenotypes C, L, and P were the most common in *H. spontaneum* (Table 2).

Although our sample of *H. vulgare* was more than twice as large as that of *H. spontaneum*, it included only nine rDNA sl phenotypes (Table 2) and 8 rDNA sl variants (Table 1), whereas the sample of *H. spontaneum* included nine phenotypes and 10 variants. Shannon's information statistic (14) ($h_s = -\Sigma f_i \ln f_i$, where f_i is frequency of rDNA sl phenotype or variant), calculated from rDNA sl phenotypic frequencies (Table 2), gives values of h_s = 0.52 and 0.85 for *H. vulgare* and *H. spontaneum*, respectively; h_s calculated from normalized rDNA sl variant frequencies (Table 1) is 0.57 for *H. vulgare* and 0.81 for *H. spontaneum*. Thus, *H. spontaneum* is more polymorphic for rDNA sl variation than *H. vulgare*, whether the variation is measured in terms of sl phenotypes or sl variants.

Table 1. Frequencies of rDNA sl variants* in *H. spontaneum* (*H.s.*) and *H. vulgare* (*H.v.*) and in the parents (P) and five † different generations of CCII

	rDNA sl variant band number												
	1	2	3	4	5	6	7	8	10	11	12	13	15
H.s.			0.08		0.20	0.08	0.92	0.40	0.20	0.12	0.12	0.12	0.04
H.v.	0.02	0.02		0.68		0.04	0.26		0.04	0.04	0.94		
P	0.04			0.61		0.07	0.32		0.07	0.07	0.89		
F_8				0.49		0.01	0.51			0.01	0.98		
F_{13}				0.48			0.52		0.01		0.99		
F_{23}				0.41		0.04	0.59			0.04	1		
F_{45}				0.47			0.55				1		
F_{53}				0.33			0.72				1		

*The rDNA sl variant frequencies reported are phenotypic frequencies; these phenotypic frequencies do not correspond to allelic frequencies for loci *Rrn1* and *Rrn2*.

†Numbers of families sampled were 79, 75, 73, 71, and 79 for generations F_8, F_{13}, F_{23}, F_{45}, and F_{53}, respectively.

Table 2. Frequencies of rDNA sl phenotypes in *H. spontaneum* (*H.s.*), *H. vulgare* (*H.v.*), and in the parents (P) and 5 different generations* of CCII

	rDNA sl phenotype†								
	B	D	F	G	H	I	J	K	L
H.s.									0.16
H.v.	0.02	0.04	0.02		0.66	0.16	0.02	0.04	0.04
P	0.04	0.07	0.04		0.57	0.14		0.07	0.07
F_8	0.03				0.49	0.47		0.01	
F_{13}					0.48	0.51			0.01
F_{23}					0.41	0.55		0.04	
F_{45}				0.01	0.45	0.54			
F_{53}				0.05	0.28	0.67			

*Numbers of families were 79, 75, 73, 71, and 79 in generations F_8, F_{13}, F_{23}, F_{45}, and F_{53}, respectively.
†Phenotypes A, C, E, M, N, O, P, and Q occurred only in *H. spontaneum*; their frequencies in our sample were 0.04, 0.28, 0.12, 0.12, 0.04, 0.04, 0.16, and 0.04, respectively.

Table 2 also shows that among the 17 different sl phenotypes observed, 8 were confined to each *H. vulgare* and *H. spontaneum*, whereas only 1 (phenotype L) was common to both. In contrast, among 13 rDNA sl variants observed in our sample, 10 were found in *H. spontaneum*, 8 in *H. vulgare*, and 5 in both. Hedrick's identity measure (15) takes values $I = 0.02$ when calculated from the data on phenotypes and $I = 0.29$ when calculated from the data on variants; thus, the combinations into which rDNA sl variants unite to produce rDNA sl phenotypes differentiate cultivated barley from its wild ancestor more distinctly than the rDNA sl variants themselves.

The Inheritance of rDNA Spacer-Length Variants. The parents chosen to study the Mendelian inheritance of rDNA sl variants were Sutter, a barley cultivar, and PI 296897, an accession of *H. spontaneum*. These parents were selected because: (*i*) they carry distinct rDNA sl variants (Sutter, phenotype H, slv-4 and slv-12; PI 296897 phenotype C, slv-7 and slv-8), and (*ii*) they differ in phenotype for two enzyme loci (esterase 1 and esterase 4) and for three easily scored morphological characters (2- vs. 6-rowed spike, V^t/v; rough vs. smooth awns, R/r; and tough vs. brittle rachis, Bt/bt). An F_2 population of 124 individuals, obtained by selfing a single F_1 hybrid plant, was grown in the greenhouse. These plants were scored for their rDNA sl phenotype and for the phenotype of the two enzyme and three morphological characters. Ninety-five of the F_2 plants were selfed, and the resulting families were scored for the two enzyme characters and for the three morphological characters to determine the genotype of their F_2 parents.

The phenotypic classes of the rDNA sl variants observed in the two parents (Sutter = P_1, PI 296897 = P_2), the F_1 hybrid, and the F_2 generation are illustrated in Fig. 3*A*. The two bands observed in Sutter, designated a (slv-12) and d (slv-4), are shown in lane 1; the two observed in PI 296897, designated b (slv-8) and c (slv-7), are shown in lane 9. All four bands appeared in the F_1 hybrid (lane 5). Nine phenotypes were observed among the 124 F_2 plants examined, as follows: the two parental phenotypes (two-banded), the F_1 phenotype (four-banded), two additional two-banded phenotypes (lanes 3 and 7), and four three-banded phenotypes (lanes 2, 4, 6, and 8). This suggests that two loci, each with two codominant alleles, govern genetic variability for rDNA in this hybrid. A test of "goodness of fit" of observed (Fig. 3*A*)-to-expected numbers, assuming independent inheritance, gave $\chi^2_{[8]} = 13.23$, $0.10 < P < 0.20$. Each of the single-locus segregations is expected to produce three phenotypic classes in proportions of 1:2:1 (Fig. 3*B*). Tests of "goodness of fit" of observed to expected numbers for the phenotypic classes aa, ab, bb and cc, cd, and dd respectively, gave $\chi^2_{[2]} = 6.1$, $0.01 < P < 0.05$, and $\chi^2_{[2]} = 2.13$, $0.50 < P < 0.70$. The significant χ^2 value for the aa, ab, bb segregation is due primarily to deficiency in the aa and excess in the bb phenotypic class, which suggests that the aa phenotype is at a disadvantage relative to the bb phenotype. This is consistent with the observation (see below) that the sl variants and/or associated loci are under selection in CCII. We conclude that the observed F_2 segregations are consistent with the hypothesis that two independently inherited loci, each with two codominant alleles (*a,b* and *c,d*, respectively), govern rDNA sl phenotypes in this hybrid. We designate these two loci *Rrn1* and *Rrn2*. Note that we specify an array of hundreds of rDNA repeat units as an allele.

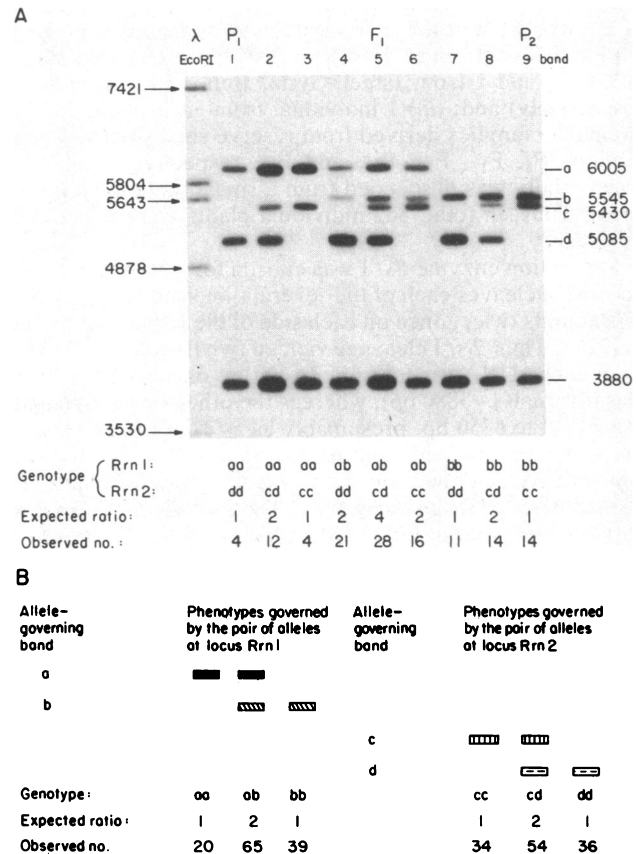

FIG. 3. (*A*) Autoradiograph of Southern blot showing the nine phenotypes, expected ratios, and numbers observed in an F_2 population derived from the hybrid between Sutter (P_1) and PI 296897 (P_2). Numbers in margins indicate the size of fragments in base pairs. (*B*) The three phenotypes governed by alleles *a* and *b* of locus *Rrn1* are diagrammed on the left and the three governed by alleles *c* and *d* of locus *Rrn2* on the right. (The separate, single-locus phenotypes have not been seen on our gels.)

These results allow three additional deductions concerning the genetic organization of the sl variants in these parents and their hybrid progeny. First, all, or nearly all, copies of sl variants 4, 7, 8, and 12 lie within loci *Rrn1* and *Rrn2* and not within loci located elsewhere in the genome. Second, each of these four sl variants is located at only one of the two rDNA loci. Thus, locus *Rrn1* contains few if any copies of variants *c* (slv-7) and *d* (slv-4), and locus *Rrn2* contains few if any copies of variants *a* (slv-12) and *b* (slv-8). *Rrn1* and *Rrn2* are therefore new gene markers in barley. Third, the nonrandom distribution of these four rDNA sl variants suggests that genetic exchange occurs much less frequently between than within nucleolus organizer regions. The same observation has been made in wheat (3), *Drosophila* (4), and the mouse (5); however, random distribution has been observed in humans (6).

Because barley rDNA hybridizes only with barley chromosomes 6 and 7 (2), we deduced that one of the two rDNA loci is located on chromosome 6 and one on chromosome 7. Wheat–barley addition lines 1, 2, 3, 4, 6, and 7 were used to test this deduction. Each of these addition lines (16) carries an homologous pair of barley chromosomes plus a complete complement of wheat chromosomes in wheat cytoplasm; addition line 5 is sterile. An autoradiograph of a Southern blot (Fig. 4) shows the rDNA sl phenotypes of the six wheat–barley addition lines and the phenotypes of the wheat and barley parents of the addition lines. The barley parent of the addition lines (Betzes, lane 7) carries the same allele-governing bands as Sutter, *a* (slv-12) and *d* (slv-4). The wheat parent (Chinese Spring, lane 8) does not carry bands *a* and *d*. Wheat–barley addition line 6 (lane 5) and line 7 (lane 6) have bands *a* and *d*, respectively. Parallel results were obtained with a second restriction enzyme, *Eco*RV (Fig. 4, lanes 8–18). Therefore, provided that both Betzes and Sutter possess the same allele in each of the two rDNA loci, *Rrn1* is located on chromosome 6 and *Rrn2* is located on chromosome 7 of barley.

Pairwise recombination values were calculated (17) for the seven segregating loci in the hybrid studied. None of the 21 values differed significantly from 0.50, which indicates that each of the seven loci is inherited independently. The *R/r* locus is located on the long arm of chromosome 7; thus, the independent segregation of the *R/r* from *Rrn2*, which also is located on chromosome 7, indicates that *R/r* is situated more than 50 crossover units from *Rrn2*. Similarly, the observed recombination value for loci *Est1* and *Bt/bt*, both located on chromosome 3, indicates that these loci are located 50 or more centimorgans from each other on this chromosome.

Temporal Changes in rDNA sl Phenotypes and rDNA sl Variants in CCII. CCII was synthesized in 1929 (18) by intercrossing 28 varieties, selected to represent a broad sample of the genetic diversity in cultivated barley, and pooling equal numbers of seeds from each of the 378 pairwise hybrid combinations to produce the initial generation of the population. CCII has since been propagated annually at Davis, California, without conscious selection, in large plots grown under standard agricultural conditions.

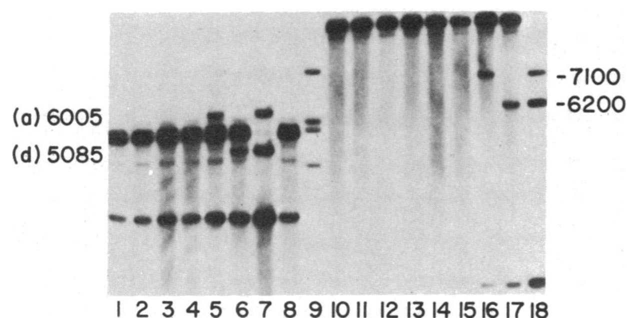

FIG. 4. Autoradiograph of a Southern blot showing rDNA sl phenotypes of wheat (lanes 8, 10, and 11), barley (lanes 7 and 18), and wheat-barley addition lines (lanes 1–6 and 12–17). DNA samples for lanes 1–8 were digested with *Sst* I and for lanes 10–18 with *Eco*RV. Lane 9 shows phage λ *Eco*RI marker fragments. Numbers indicate the size in base pairs of the indicated fragments.

Table 2 shows that the 28 parents from which CCII was synthesized included seven among the eight rDNA sl phenotypes that have been observed in *H. vulgare* (phenotype J was absent). Phenotype H was present in much higher frequency (0.57) in the parents of CCII than the next most frequent phenotype (I, 0.14); the five remaining phenotypes were all infrequent or rare (≤0.07). Thereafter, phenotype I increased in frequency until, in generation 53, it made up more than two-thirds of the population. The increase of phenotype I was at the expense of phenotypes D, F, K, and L, which disappeared from the population by the middle generations, and also was at the expense of phenotype H, which ultimately decreased to less than half its original frequency.

Among the phenotypes that were observed in the various generations of CCII, only one was not observed in the parents; this was phenotype G, which was first seen in generation F_{45}. The banding pattern of phenotype G suggested that it might represent the heterozygote between the H and I homozygotes. To test this, we progeny-tested a G individual; it produced a family that segregated into parental (H and I) and heterozygote G phenotypes. We also assayed 26 F_2 individuals from an F_1 hybrid obtained by crossing varieties with phenotypes H and I. The F_2 included 5 H/16 G/5 I phenotypes; the fit of observed to expected numbers, assuming a 1:2:1 segregation, was good ($\chi^2_{[2]} = 1.39$, $0.50 < P < 0.75$). This study showed further that (*i*) phenotypes H and I are both fixed for slv-12 at locus *Rrn1*, and (*ii*) that H is fixed for slv-4 and I is fixed for slv-7, both at locus *Rrn2*.

The frequencies of rDNA sl variants in the parents and various generations of CCII are given in Table 1. slv-12, which was frequent (0.89) in the parents of CCII, had become fixed (1.00) in the population by generation F_{23}; this variant, although common in *H. vulgare* was infrequent in *H. spontaneum*. slv-4 was common (0.61) in the parents of CCII; however, by generation F_{53}, it had decreased to about half its original frequency, whereas slv-7 had more than doubled in frequency. By generation F_{45} only three variants, slv-12, slv-7, and slv-4, remained in the population; all others had disappeared. By assuming that slv-12 is exclusively an allele of *Rrn1* and slv-4 and slv-7 are exclusively alleles of *Rrn2*, the genetic composition of CCII in generation 53 can be inferred to be as shown in Table 3. Thus, variant frequencies in generation F_{53} are: *Rrn1-a* (slv-12) = 1.000; *Rrn2-c* (slv-7) = 0.695; *Rrn2-d* (slv-4) = 0.305.

The above results show that substantial changes in frequency occurred in CCII over generations for each of the seven rDNA sl phenotypes and seven rDNA sl variants that were contributed to CCII by its parents. Genetic drift, migration, and mutation are, for the following reasons, unlikely candidates among the evolutionary factors which might be responsible for these changes: (*i*) observed changes in frequency were more than an order of magnitude larger than the maximum cumulative effect that genetic drift is expected to produce in a population as large as CCII (>15,000 reproduc-

Table 3. Genetic composition of CCII in generation 53

sl phenotypes	sl variant band no.	*Genotype* *Rrn1*, *Rrn2*	Frequency
H	4, 12	*aa*, *dd*	0.28
I	7, 12	*aa*, *cc*	0.67
G	4, 7, 12	*aa*, *cd*	0.05

ing adults per generation); (*ii*) studies with allozymes have provided direct evidence that little if any migration occurred into CCII (19); (*iii*) the only phenotype that arose *de novo* in CCII (phenotype G) can be accounted for on the basis of hybridization between two other phenotypes in the population. This indicates that no novel sl variants attributable to mutation appeared in CCII during more than 50 generations of propagation in large populations. Therefore, by elimination, we identify selection as the evolutionary force responsible for the directional changes that occurred in CCII. The main feature of directional change was the large increase in frequency of phenotype I, and the two sl variants (slv-7 and slv-12) that occur in this phenotype. Estimates of the selective values, made by computer simulation, show that individuals with phenotype I had an average selective advantage of ≈10% over the other phenotypes in the population in the period from synthesis to generation F_8 and also from generation F_{45} to F_{53} but little advantage in the intermediate generations. Estimates of selective values for slv-12 indicate that its average selective advantage was ≈30% from synthesis to generation F_{23}, when it became fixed. The selective advantages of slv-7 were parallel to those of phenotype I—i.e., large (10%) from synthesis to generation F_8, small in the intermediate generations, and again large (6%) from generation F_{45} to F_{53}.

We conclude that substantial directional selection took place during many generations. But this does not establish that the sl variants, or the phenotypes in which they are combined, were themselves under selection. In populations that reproduce by mixed selfing and random mating, such as CCII, the mating system imposes a correlational structure on the entire multilocus array such that each locus is influenced by the selective effects of all other loci, including loci located on different chromosomes (20). Thus, what is measured by changes in sl variant frequencies at loci *Rrn1* and *Rrn2* are not only the selective effects of these two loci themselves but also the effects of the flow of selection throughout the entire genome. Because the effects of these two marker loci are confounded not only with those of loci closely linked with them on chromosomes 6 and 7 but also with unlinked loci located throughout the genome, the loci specifically responsible for the selection cannot be identified. We also note that the heterozygous phenotype G, which first appeared in generation F_{45}, more than tripled in frequency by generation F_{53}. This rapid increase in frequency suggests that phenotype G holds a selective advantage over its corresponding homozygous phenotypes H and I. Moreover, the observed frequency of phenotype G in generation F_{53} relative to phenotypes H and I, gives an estimate of the Fixation index, $\hat{F} = 1 - G/2(H + \frac{1}{2}G)(I + \frac{1}{2}G) = 0.88 \pm 0.058$, a value substantially smaller than the theoretical inbreeding coefficient given by $F_e = s/1 + t = 0.99$ [CCII mates by 0.994 of self fertilization (s) and 0.006 of random outcrossing ($t = 1 - s$)]. Heterozygotes (phenotype G) are thus in substantial excess over expectations based on the assumption that phenotypes G, H, and I are selectively neutral. We conclude that substantial selection has taken place in CCII, but once more we cannot attribute the observed excess of heterozygotes to the rDNA loci alone because the excess may reflect an "entire-genome heterosis" transmitted by the correlational structure imposed on the population by the mating system of predominant self-fertilization.

We are grateful to Robert Bernatzky for his advice and assistance. This work was supported in part by United States Department of Agriculture-Science and Education Administration Grant 59-2063-1-1-735-0 and in part by National Institutes of Health Grant GM 32429.

1. Long, E. O. & Dawid, I. B. (1980) *Annu. Rev. Biochem.* **49**, 727–764.
2. Appels, R., Gerlach, W. L., Dennis, E. S., Swift, H. & Peacock, W. J. (1980) *Chromosoma* **78**, 293–311.
3. Appels, R. & Dvorak, J. (1982) *Theor. Appl. Genet.* **63**, 337–348.
4. Boncinelli, E., Borghese, A., Graziani, F., La Mantia, G., Manzi, G., Mariani, C. & Simeone, A. (1983) *Mol. Gen. Genet.* **189**, 370–374.
5. Arnheim, N., Treco, D., Taylor, B. & Eicher, E. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4677–4680.
6. Krystal, M., D'Eustachio, P., Ruddle, F. H. & Arnheim, N. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5744–5748.
7. Coen, E. S., Thoday, J. M. & Dover, G. (1982) *Nature (London)* **295**, 564–568.
8. Murray, M. G. & Thompson, W. F. (1980) *Nucleic Acids Res.* **8**, 4321–4325.
9. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
10. Gerlach, W. L. & Bedbrook, J. R. (1979) *Nucleic Acids Res.* **7**, 1869–1885.
11. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **133**, 251–273.
12. Miniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
13. Kahler, A. L. & Allard, R. W. (1970) *Crop Sci.* **10**, 444–448.
14. Bowman, K. O., Hutcheson, K., Odum, D. P. & Shenton, L. R. (1971) *Stat. Ecol.* **3**, 315–359.
15. Hedrick, P. W. (1971) *Evolution* **25**, 276–280.
16. Islam, A. K. M. R., Shepherd, K. W. & Sparrow, D. H. B. (1981) *Heredity* **46**, 161–174.
17. Hakim-Elahi, A. (1984) *J. Hered.* **75**, 150–151.
18. Harlan, H. V. & Martini, M. L. (1929) *J. Am. Soc. Agron.* **21**, 487–490.
19. Allard, R. W., Kahler, A. L. & Weir, B. S. (1972) *Genetics* **72**, 489–503.
20. Clegg, M. T., Kahler, A. L. & Allard, R. W. (1978) *Genetics* **89**, 765–792.