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Variation in the ribosomal DNA intergenic spacer of a maize population mass-selected for high grain yield *

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Summary. Variation in the intergenic spacer of ribosomal DNA (rDNA) was detected among individual plants of the open-pollinated maize variety 'Hays Golden' and populations derived from this variety. rDNA intergenic spacer-length variants were detected at approximately 200 bp intervals, consistent with the number of 200 bp subrepeats as the basis for this variation. Inheritance data revealed that more than one spacer-length class may be present on an individual chromosome. Fourteen different predominant rDNA intergenic spacer hybridization fragment patterns were detected. C-29, a population developed by 29 cycles of mass-selecting Hay Golden for high grain yield, exhibited a significant change in rDNA intergenic spacer hybridization fragment pattern composition in comparison to Hays Golden. This change included a reduction in frequency of the shortest predominant space-length variant (3.4 kb) and an increase in a 5.2-kb hybridization fragment. I-31, a population developed through thermal neutron irradiation of Hays Golden and 31 generations of mass selection for high grain yield, did not exhibit a significant change in overall rDNA intergenic spacer composition. I-31 did exhibit an increase in frequency of the 5.2-kb hybridization fragment and a significant change in two specific hybridization fragment patterns that had also changed in C-29. These data, particularly for the C-29 population, suggest that rDNA intergenic spacer-length variants and/or associated loci were influenced by selection.

Key words: Ribosomal DNA – Ribosomal RNA genes – Intergenic spacer – Maize population

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Introduction

Evaluation of change in the genetic composition of natural and breeding populations is a fundamental aspect of population biology. The development of molecular techniques has enhanced the precision with which genetic variation in populations can be examined. The populations developed in the mass selection experiments involving the open-pollinated 'Hays Golden' maize (*Zea mays* L.) variety have been studied for changes in traits such as yield, plant height, and maturity (Gardner 1961, 1968, 1969), examined for changes in isozyme frequencies (Kahler et al. 1984; Pollak et al. 1984; Kling et al. 1987), and are also suitable for analysis at the molecular level. In this study we evaluated variation in the ribosomal RNA genes of Hays Golden and populations developed through mass selection for high grain yield.

The ribosomal genes are present in tandemly repeated arrays at the nucleolus organizer (NOR) on chromosomes *6S* of maize (Phillips et al. 1971). Ribosomal RNA (rRNA) is transcribed as a precursor that is processed into the mature *17S*, *5.8S*, and *26S* rRNAs responsible for translation of RNA messages. Each repeat of ribosomal DNA (rDNA) contains a single rRNA transcription unit as well as an intergenic spacer (IGS) region. This region has also been termed the nontranscribed spacer region. However, work in animal systems has indicated that transcription does occur in this region, generating short-lived products (for review, Baker and Platt 1986). In many species of both plants and animals there is considerable variation between individuals for rDNA repeat length (*Xenopus laevis*, Reeder et al. 1976; *Drosophila melanogaster*, Coen et al. 1982b; *Triticum aestivum*, Appels and Dvorak 1982; *Homo sapiens*, Ranzani et al. 1984; *Hordeum vulgare*, Saghai-Maroof et al. 1984; Vicia faba, Rogers et al. 1986). The variation in rDNA repeat

length usually results from the presence of differing numbers of short repeated segments within the IGS region (for review, Rogers and Bendich 1987). These short repeated sequences have been shown to be associated with functional aspects of rDNA gene expression in *Xenopus laevis*, acting as enhancers of polymerase I activity (Moss 1983; Reeder et al. 1983). In maize, it has been proposed that the spacer subrepeats may play an important role in regulating maize rRNA transcription (McMullen et al. 1986; Tolocyzki and Feix 1986).

Changes in rDNA IGS composition have been observed in *Hordeum vulgare*, *Triticum dicoccoides*, and *Drosophila melanogaster* populations that have undergone natural or directional selection. Saghai-Maroof et al. (1984) observed that substantial directional selection occurred for specific rDNA spacer-length variants in a barley composite population during 54 generations of natural selection. Flavell et al. (1986) observed that rDNA spacer diversities of 12 separate natural populations of *Triticum dicoccoides* were significantly correlated and predictable in terms of climatic variables. Cluster et al. (1987) observed changes in rDNA spacer-length composition of *Drosophila melanogaster* populations that had undergone recurrent cycles of selection for development rate.

In this paper we report on: (1) the extent of rDNA intergenic spacer-length polymorphism present in Hays Golden; (2) the segregation of selected spacer-length variants in two F_2 populations; and (3) the comparison of rDNA spacer-length composition of Hays Golden and derivative populations that have undergone up to 31 cycles of mass selection for yield.

Materials and methods

Genetic materials

All plants evaluated for rDNA spacer-length variation originated from open-pollinated Hays Golden (HG). Random kernels were obtained from unselected HG, three generations (C10, C20, and C29) of the control population, and from the irradiated population (I-31). These populations had been improved by 10, 20, 29, and 31 cycles of mass selection for increased grain yield, respectively. The HG samples was HG81; this represents a 1981 increase of Hays Golden. The mass selection procedure and thermal neutron treatment of the irradiated population have been described by Gardner (1961, 1968, 1969). Several crosses of individual Hays Golden plants × individual C-29 plants were made. The parents and sample F_1 plants from three of these crosses were analyzed for rDNA spacer hybridization fragment patterns. Two F_2 populations were developed from two different F_1 plants sampled from one of these crosses.

The number of random individual plant rDNA samples was 81, 23, 23, 73, and 63 in HG, C-10, C-20, C-29, and I-31, respectively. A small number of plants was sampled from generations C-10 and C-20, since the primary purpose of investigating these two generations was to search for spacer hybridization fragment patterns not detected in the other generations sampled. The samples evaluated from the two F_2 populations consisted of 22 and 16 plants, respectively. The purpose of evaluating the F_2 populations was primarily to investigate the contribution of different chromosome 6 homologues to the overall spacer hybridization fragment patterns and not to perform a full inheritance study. In total, 303 individual rDNA samples were evaluated.

DNA isolation

Total cellular DNA was isolated from individual seedlings, except for use of mature leaf tissue from the parents and F_1's associated with the segregation analysis. One gram of leaf tissue was homogenized with mortar and pestle in 13 ml of extraction buffer [2 *M* NaCl, 10 m*M* *TRIS* (pH 8.0), 10 m*M* EDTA (pH 8.0), 1% SDS], then rehomogenized after addition of 15 ml of chloroform. The emulsion was centrifuged at 10,000 × *g* for 10 min, the aqueous phase was removed, placed on ice, and then precipitated with 0.35 vol. isopropanol. The nucleic acid precipitate was recovered by centrifugation, rinsed with 70% ethanol, and resuspended in 1 ml TE [10 m*M* *TRIS*, 1 m*M* EDTA (pH 8.0)]. To the solution 7.5 *M* sodium acetate was added to make it 0.3 *M*. One volume of isopropanol was added and the precipitate was spooled out immediately and resuspended in TE.

Restriction enzyme digestion, blotting, and hybridization

Five micrograms of DNA was digested to completion with 15 units of restriction endonuclease SstI for 8–16 h at 37 °C. SstI was selected since there are SstI recognition sites that essentially generate a spacer-specific restriction fragment (Fig. 1). DNA restriction fragments were fractionated by electrophoresis in 0.7% agarose gels. Restriction endonuclease digestions and gel electrophoresis were performed essentially as outlined by Maniatis et al. (1982). To preclude incomplete digestion, additional quantities of restriction enzyme were added to selected samples, and lambda was added to some digests and analyzed for appropriate digestion. Transfer of DNA to nylon filters and Southern hybridization analyses were performed according to conditions outlined by Reed and Mann (1985). The filters were hybridized to ^{32}P-labeled pZmrs-1, a 3.2-kb clone of the maize spacer region provided by B. Hunter and I. Rubenstein, Department of Genetics and Cell Biology, University of Minnesota (Fig. 1). Nick-translation was performed essentially as described by Rigby et al. (1977) and autoradiography was done at −70 °C with Dupont Lightning Plus intensifying screens.

Evaluation of rDNA spacer variants

Southern blots were visually scored for presence of major hybridization fragments to characterize restriction fragment length polymorphisms. The frequencies of the major individual hybridization fragments detected in Hays Golden and the im-

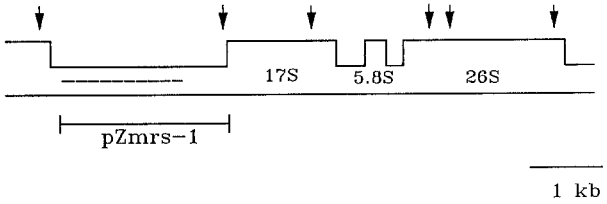

Fig. 1. A single repeat unit of a maize ribosomal RNA gene. Sst I restriction endonuclease cleavage sites are indicated by *arrows*. *Dashed lines* in intergenic spacer region represent the approximately 200-bp tandem subrepeats (McMullen et al. 1986; Tolocyzki and Feix 1986)

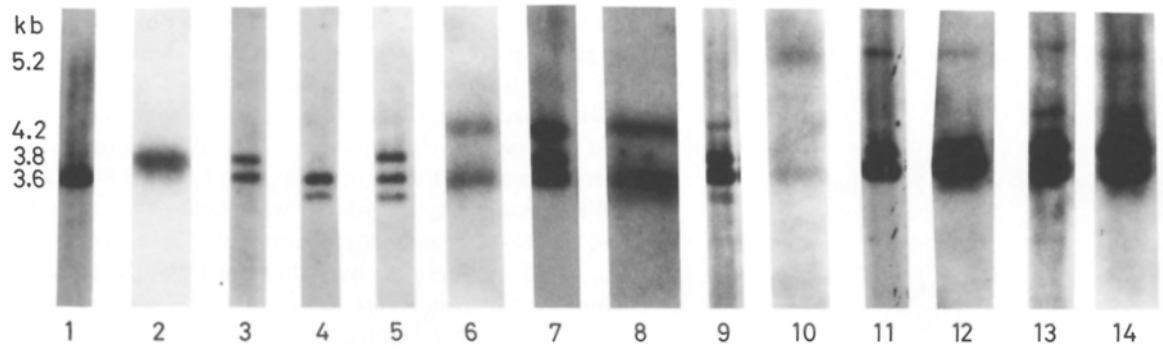

Fig. 2. Summary of predominant hybridization fragment patterns detected among individual plants evaluated from Hays Golden. DNA was isolated from individual seedlings, digested with Sst I, fractionated by electrophoresis, transferred to nylon membrane, and hybridized with ^{32}P-labelled rDNA spacer clone pZmrs-1, and autoradiography was performed. Samples illustrated were taken from autoradiograms of Southern blots from different-sized gels with differing durations of electrophoresis. Photographic enlargement to standardize samples was performed accordingly

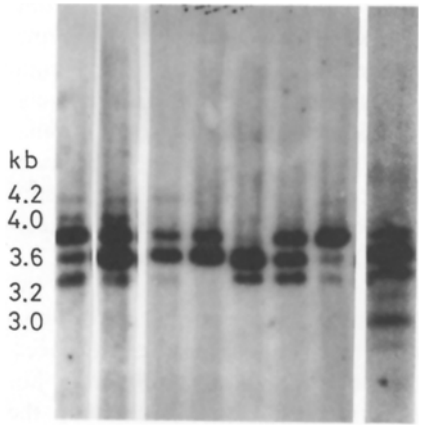

Fig. 3. Autoradiographs of Southern blots that illustrate the copy number variability for some rDNA spacer-length variants and the presence of rDNA spacer-length variants revealed upon long exposure time. DNA was isolated from individual seedlings, digested with Sst I, fractionated by electrophoresis, transferred to nylon membrane, and hybridized with ^{32}P-labelled rDNA spacer clone pZmrs-1, and autoradiography was performed

proved populations were calculatd and compared by Chi-square analysis. The frequencies for each of the different hybridization fragment patterns were compiled for each population and compared by Chi-square analysis. Data from two or more similar classes of hybridization fragment patterns were collapsed together in some cases to increase expected numbers in each class of five or more (Cochran 1954). For analysis of F_2 populations, Chi-squares analyses were adjusted for small numbers (Cochran 1954).

Results

Ribosomal DNA intergenic spacer-length variation in Hays Golden and improved populations

Ribosomal DNA spacer-length variation was detected among individual plants sampled from Hays Golden and the improved populations. Primary hybridization of intergenic spacer probe pZmrs-1 occurred to DNA fragments of approximately 3.4, 3.6, 3.8, and 4.2 kb (Fig. 2). Lower intensity hybridization was detected for a 5.2-kb fragment. This fragment results from loss of the SstI restriction site in the external transcribed spacer region, and it is composed of the 3.6-kb spacer-length variant and 1.6 kb of the 5′ end of the *17 S* ribosomal RNA gene (Fig. 1). Since the entire IGS region is present in this fragment, the lower intensity hybridization signal suggests that only a portion of the repeats has lost the SstI restriction site that generates the 5.2-kb fragment. Longer exposure of some autoradiograms revealed minor hybridization signals at 3.0, 3.2, and 4.0 kb (Fig. 3). The low intensity of these hybridization signals suggests that these particular spacer-length variants are present in considerably lower copy number than the predominant spacer-length variants. The hybridization data reveal that a series of seven spacer-length variants differing by approximately 200-bp increments from 3.0 to 4.2 kb are present in the Hays Golden populations.

The predominant spacer hybridization fragment patterns detected in individual plants of Hays Golden are summarized in Fig. 2. The 14 different spacer hybridization fragment patterns exhibit a range of complexity. Spacer hybridization fragment patterns 1 and 2 are composed of a single hybridization band, indicating that only a single spacer-length variant is present. Other hybridization fragment patterns are composed of multiple hybridization bands, which can be attributed to the presence of more than one spacer-length variant.

Organization of intergenic spacer-length variants

Plants with spacer hybridization fragment patterns 7 (P_1) and 4 (P_2) were selected as parents (cross 1) of an F_2 population for the segregation analysis, because together they include the predominant 3.4-, 3.6-, 3.8-, and 4.2-kb

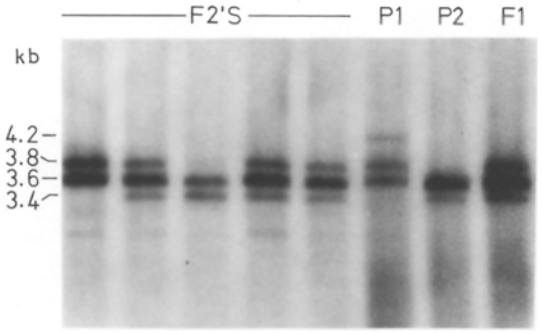

Fig. 4. Autoradiograph of a Southern blot that illustrates the inheritance of selected maize rDNA spacer-length variants. DNA from the two parents, P_1 and P_2, the F_1, and samples of F_2 plants was isolated and cleaved with Sst I, separated by electrophoresis, transferred to nylon membrane, and hybridized with ^{32}P-labelled rDNA spacer clone pZmrs-1

Table 1. Expected ratios and numbers observed on two F_2 populations derived from the F_1 of parents with different hybridization fragment patterns. F_2 no. 1 and no. 2 are derived from F_1's with hybridization pattern nos. 5 and 7, respectively

Expected ratio	1	2	1
Genotype	aa	ab	bb
Observed numbers			
F_2 no. 1	6	14	2
Genotype	cc	cd	dd
Observed numbers			
F_2 no. 2	6	6	4

Tests for goodness of fit of observed to expected numbers: F_2 no. 1, $\chi^2 = 2.20$, $0.25 < P < 0.50$; F_2 no. 2, $\chi^2 = 0.96$, $0.5 < P < 0.75$

Table 2. Estimates of frequencies of rDNA intergenic spacer hybridization fragments in Hays Golden and improved populations

Size (kb)	Population		
	HG	C-29	I-31
5.2	0.14	0.44	0.27
4.2	0.17	0.12	0.22
3.8	0.76	0.73	0.77
3.6	1.00	0.99	1.00
3.4	0.62	0.22	0.57

spacer-length variants (Fig. 4). The two sets of parents associated with the other HG × C29 F_1's evaluated (crosses 2 and 3) both exhibited spacer hybridization fragment pattern 3 for one parent (P_1) and pattern 5 for the other parent (P_2) (data not shown). Evaluation of three separate F_1 plants from different kernels of cross 1 revealed hybridization fragment patterns 3, 5, and 7, respectively (data not shown). Evaluation of separate F_1 plants from crosses 2 and 3 detected spacer hybridization fragment patterns 3, 4, and 5 (data not shown).

These findings indicate that for cross 1, the 4.2- and 3.4-kb spacer-length variants were heterozygous in P_1 and P_2, respectively, present on only one homologue of chromosome 6. For crosses 2 and 3, the 3.8-kb and 3.4-kb spacer-length variants were heterozygous in P_2 and the 3.8-kb spacer-length variant was heterozygous in P_1. The F_1 plants with hybridization fragment patterns 5 and 7 from cross 1 were selfed and the F_2 were progeny evaluated. Figure 4 illustrates the spacer hybridization fragment patterns of the parents, the F_1 with hybridization fragment pattern 5, and a sample of the corresponding F_2 population. Figure 4 also illustrates that two different spacer-length variants were inherited as a single, stable allele in the F_2 progeny. One allele contained the 3.4- and 3.6-kb variants, the other contained the 3.6- and 3.8-kb variants. This is supported by scanning densitometry that indicated that when three spacer-length variants were present in a single lane, the 3.6-kb spacer-length variant exhibited a greater hybridization intensity relative to the 3.4- and 3.8-kb variants. The two F_2 populations evaluated from F_1's with hybridization fragment patterns 5 and 7, respectively, both produced a reasonable fit to a 1 : 2 : 1 segregation for the predicted spacer-length patterns (Table 1).

The previous result indicates that some spacer hybridization fragment patterns represent a heterozygous condition for chromosomes containing different spacer-length variants, while other patterns represent a homozygous condition for chromosomes containing the same spacer length variants. Spacer hybridization fragment pattern 5 could be generated by crossing parents with the spacer-length variants present in spacer hybridization fragment patterns 3 and 4. Other spacer hybridization fragment patterns, such as 1 or 2, appear to indicate a homozygous condition for a particular spacer-length variant. The relative hybridization intensity of the different spacer-length classes may indicate that an individual variant is present in significant numbers on one or both homologues. However, since there is also considerable copy number variation present for spacer-length variants in some plants (Fig. 3), determination of a heterozygous or homozygous condition requires evaluation of progeny of the plants in question.

Frequency of intergenic spacer hybridization fragments

The frequencies of the predominant intergenic spacer-length variants in Hays Golden and improved populations are summarized in Table 2. The 3.6-kb spacer-length variant was present in all but one plant sampled from these populations and, therefore, no significant frequency changes for this variant occurred between Hays Golden and either improved population (Table 3). Simi-

larly, the 3.8-kb spacer-length variant, present in approximately 75% of the plants surveyed, did not undergo a frequency change between Hays Golden and either improved population. The 3.4-kb spacer-length variant was present in 62% of the plants surveyed from Hays Golden and decreased significantly to a 22% frequency in the C-29 population. The frequency of the 3.4-kb spacer-length variant was not significantly lower for the I-31 population than for Hays Golden. The 4.2-kb spacer-length variant was detected in 17% of plants surveyed from Hays Golden and there was not a significant frequency shift of this variant in either improved population. The 5.2-kb hybridization fragment was present in 14% of the Hays Golden plants surveyed. The frequency of this hybridization fragment increased significantly in both improved populations, exhibiting a greater increase in C-29 than I-31.

Frequency of intergenic spacer hybridization fragment patterns

The frequencies of the different spacer hybridization fragment patterns present in Hays Golden and improved populations are listed in Table 4. The tests of homogeneity of rDNA spacer hybridization pattern composition of Hays Golden and the improved populations are listed in Table 5. The C-29 population exhibited a significant change in spacer hybridization pattern composition in comparison to Hays Golden. The I-31 population did not exhibit a change in spacer hybridization fragment pattern composition. The C-29 spacer composition was also significantly different from I-31 composition.

Frequency changes for just a few of the spacer-length hybridization fragment patterns contributed largely to the significant overall change in spacer composition between Hays Golden and C-29. Spacer hybridization fragment patterns 4 and 5 were lower in frequency and spacer hybridization fragment pattern 11 was higher in frequency in the C-29 population in comparison to Hays Golden. Since spacer hybridization patterns 4 and 5 contain the 3.4-kb spacer-length variant and spacer hybridization pattern 11 does not, the change in the frequency of plants with these hybridization patterns reduced the frequency of the 3.4-kb spacer-length variant in C-29. The increase in spacer hybridization fragment pattern 11 also contributed to the increase of the 5.2-kb hybridization fragment in C-29. Although I-31 did not exhibit a significant change in overall spacer hybridization fragment composition, some individual spacer hybridization fragment patterns underwent frequency changes. Spacer hybridization fragment pattern 5 decreased in frequency ($\chi^2 = 5.17$, $P < 0.025$) and spacer hybridization fragment pattern 11 increased in frequency ($\chi^2 = 4.06$, $P < 0.05$), both similar to changes observed in C-29, but the changes were of a smaller magnitude.

Some spacer hybridization fragment patterns that were detected in Hays Golden in very low frequency were not observed in one or both of the selected populations. These spacer hybridization fragment patterns contained either the 4.2-kb spacer-length variant or the 5.2-kb hybridization fragment, both present in lower frequency in Hays Golden than the other hybridization fragments. These low frequency spacer hybridization fragment patterns appear to be composed of spacer-length variants that occur together rarely because of the low frequency of chromosomes that contain the specific-length classes required to generate these hybridization patterns. The absence of these spacer hybridization fragment patterns

Table 3. Chi-square values and probability values for tests of homogeneity of rDNA intergenic spacer hybridization fragment frequencies of Hays Golden and improved populations

Size (kb)	Contrast		
	HG vs C-29	HG vs I-31	C-29 vs I-31
5.2	18.05** <0.005	4.06* <0.05	4.17* <0.05
4.2	0.74 <0.50	0.55 <0.50	2.36 <0.25
3.8	0.31 <0.75	0.03 <0.90	0.48 <0.50
3.6	0.01 <0.95	0.00 <0.99	0.01 <0.95
3.4	23.97** <0.005	0.31 <0.75	17.75** <0.005

*,** Significant at the 0.05 and 0.01 probability levels, respectively

Table 4. Frequencies of rDNA intergenic spacer hybridization fragment patterns of Hays Golden and improved populations

Population	Spacer hybridization fragment pattern													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HG	0.06	0.00	0.14	0.14	0.38	0.01	0.10	0.01	0.02	0.01	0.04	0.06	0.02	0.00
C-29	0.18	0.01	0.16	0.01	0.11	0.03	0.04	0.00	0.00	0.06	0.30	0.04	0.00	0.06
I-31	0.05	0.00	0.19	0.14	0.21	0.00	0.02	0.03	0.10	0.00	0.13	0.06	0.05	0.03

Table 5. Chi-square values and probability values for tests of homogeneity of rDNA intergenic spacer hybridization fragment pattern composition in Hays Golden and improved populations

	χ^2	P
Hays Golden vs C-29	42.99**	<0.005
Hays Golden vs I-31	8.23	<0.250
C-29 vs I-31	20.30**	<0.005

** Significant at the 0.01 probability level

may possibly be due to sampling error rather than actual disappearance from the population.

The plants evaluated from generations C-10 and C-20 revealed no unique spacer hybridization fragment patterns. Spacer hybridization fragment pattern 2 was detected in C-10; this supports the authenticity of this unique hybridization pattern, since it was detected in only one plant of the C-29 population. Spacer hybridization fragment pattern 2 consists of only the 3.8-kb spacer-length variant, and is apparently a homozygote of chromosomes with this spacer-length class predominant. Additionally, C-10 and C-20 samples were evaluated for frequency shifts of the predominant spacer-length variants. This analysis suggests that the 3.4-kb variant may have decreased in a consistent manner during the successive cycles of mass selection, exhibiting frequencies of 0.62 in HG, 0.52 in C-10, 0.26 in C-20, and 0.22 in C-29. In contrast, the 5.2-kb hybridization fragment may have undergone a frequency increase in the later generations, since its frequency in C-10 and C-20 was similar to Hays Golden, while in C-29 the frequency was considerably higher. Since sample size was small (23) for generations C-10 and C-20, the possibility of sampling error should be noted.

Discussion

The results in this paper reveal the considerable spacer-length variation in the rDNA repeats of Hays Golden. Our observation of spacer-length differences at approximately 200-bp increments suggests that the number of 200-bp subrepeats (McMullen et al. 1986; Tolocyzki and Feix 1986) is the primary basis for spacer-length variation in maize. Sequencing analysis of the spacer region of maize inbred Black Mexican Sweet detected ten 200-bp subrepeats (McMullen et al. 1986). Southern analysis with spacer probe pZmrs-1 of Black Mexican Sweet DNA digested with restriction enzyme SstI produces a 3.6-kb hybridization fragment (Jupe et al. 1988) similar to Hays Golden. The 3.6-kb spacer-length variant detected in Hays Golden is therefore inferred to contain ten 200-bp subrepeats. Correspondingly, the predominant 3.4-kb, 3.8-kb, and 4.2-kb spacer-length variants may contain 9, 11, and 13 200-bp subrepeats, respectively. The presence of low-copy-number spacer-length variants of approximately 3.0 kb, 3.2 kb, and 4.0 kb is also consistent with number of 200 bp subrepeats as the primary basis for spacer-length variation.

The 5.2-kb hybridization fragment detected in Hays Golden and improved populations has also been observed in teosinte, *Tripsacum*, Black Mexican Sweet, and inbreds B37 and B73 (Jupe et al. 1988). The low intensity 5.2-kb hybridization signal detected in these lines is similar in intensity to that of Hays Golden. This lower intensity signal suggests that this restriction site variant is found in only a small percentage of the rDNA repeats.

Comparison of the spacer hybridization fragment patterns detected in Hays Golden suggests that some hybridization fragment patterns can be easily explained as homozygotes or heterozygotes of different spacer-length variant alleles. These observations are consistent with the Mendelian inheritance patterns of spacer-length variants observed in barley (Saghai-Maroof et al. 1984). Our segregation data suggests that two different spacer-length classes are present on an individual chromosome. Observation of the various spacer hybridization fragment patterns also suggests that some chromosomes may contain three of the predominant spacer-length classes. For example, spacer hybridization fragment pattern 9 exhibits four spacer-length classes; since the 3.6-kb length class is present in 99.24% of plants evaluated, both chromosomes that comprise spacer-length hybridization fragment pattern 9 have a high probability of possessing the 3.6-kb length class. It follows that one chromosome must contain a total of three length classes to generate the four-length class pattern. If the low-copy-number length classes are considered, four different length classes may be present on an individual maize chromosome.

The distribution of spacer-length variants in Hays Golden is clustered around the 3.6-kb spacer-length variant. The 3.6-kb spacer-length variant is present in nearly all plants, the 3.4-kb and 3.8-kb spacer-length variants are both present at intermediate frequencies, and the 4.2-kb spacer-length variant is present at a lower frequency. Inclusion of the lower copy spacer-length variants reveals there are three detectable spacer-length classes longer (3.8, 4.0, 4.2 kb) and three shorter (3.0, 3.2, 3.4 kb) than the 3.6-kb length class. This type of clustering of spacer-length variants has also been observed in *Vicia faba* (Rogers et al. 1986) and wild populations of *Triticum dicoccoides* (Flavell et al. 1986).

The concept of converted evolution of rRNA genes has support from the composition of spacer-length variants by a large number of repeat units of the same length (Coen et al. 1982a). Tandem arrays of rRNA genes can be homogenized for a particular length variant by cycles

of unequal crossing-over between complete repeat units or gene conversion (Smith 1976; Szostak and Wu 1980). In contrast, unequal crossing-over between tandem arrays of intergenic subrepeats produces rDNA repeat units of differing lengths (Coen et al. 1982a). The homogeneity of repeat lengths observed in this population may be due to greater frequency of unequal crossovers between whole repeat units than between 200-bp intergenic subrepeats. Our observation of a distribution of spacer lengths around the most frequent spacer-length class also suggests that unequal crossovers between 200 bp intergenic subrepeats are the basis for spacer-length variability. However, the 4.2-kb spacer variant is generally present in a much higher copy number than the 4.0 kb spacer variant, which is generally detected only through long autoradiogram exposure. If unequal crossing-over alone were responsible for the distribution and composition of spacer-length variants, the 4.0-kb spacer variant would be expected to be present in copy number similar to the 4.2-kb spacer variant. This suggests that other mechanisms or selection may have affected the organization of the rDNA intergenic spacers present in Hays Golden.

The change in spacer hybridization fragment pattern composition of the C-29 population in comparison to Hays Golden is consistent with the findings of Saghai-Maroof et al. (1984). They reported changes in spacer hybridization fragment pattern composition of a barley composite (CCII) that had undergone natural selection. Comparison of advanced generations with the original barley composite revealed one spacer-length hybridization fragment pattern increased extensively, another decreased considerably, and all others originally present disappeared from the population. Saghai-Maroof et al. (1984) concluded that spacer-length variants and/or associated loci were under selection in this barley composite. They were not able to determine whether selection of rDNA spacer-length variants alone was responsible for the frequency changes.

Comparison of results from the Hays Golden and Barley CCII populations requires consideration of the fundamental differences between the two selection studies. The barley composite was created with diverse parents from the world's barley-growing regions. Some parents may not have been adapted to the growing environment in Davis/CA. The change observed in the frequency of spacer-length hybridization fragment patterns, particularly those that disappeared from the population in the early generations, may have been a result of close association with nonadapted loci rather than selection against the spacer-length variants. In contrast, Hays Golden is considerably more homogeneous than the original barley composite and is adapted to the western Corn Belt environment. During the cycles of mass selection, change in spacer hybridization fragment pattern composition due to association with nonadapted loci was less likely to have been a factor. However, directional selection for yield was performed for Hays Golden rather than natural selection for barley CCII. Therefore, spacer composition changes in Hays Golden may be a result of selection of closely associated favorable loci and not selection of rDNA spacer variants. In this event, the spacer hybridization fragment patterns may serve as markers of favorable alleles.

An important component of the change observed in rDNA intergenic spacer composition of the C-29 population was the reduction in frequency of the shortest predominant spacer-length class (3.4-kb). Reduction in frequency of shorter spacer-length variants was reported in the *Drosophila melanogaster* population that underwent selection for rapid preadult development rate (Cluster et al. 1987). These shorter spacer-length variants were considered to contain fewer copies of the intergenic subrepeats. These subrepeats have been shown to contain duplicate gene promoter elements that enhance the rate of rDNA transcriptional efficiency in *Xenopus laevis* (Moss 1983; Reeder et al. 1983). In the *Drosophila melanogaster* selection study it was suggested that longer spacer elements may be at an advantage or more favorable due to greater rDNA transcriptional efficiency. For maize, although elements similar to transcription initiation sites have been detected in the 200-bp subrepeats (Toloczyki and Feix 1986; McMullen et al. 1986), it is presently not possible to suggest a relationship between the reduction of the short spacer-length variant in C-29 and transcriptional efficiency.

The I-31 population did not exhibit a change in spacer hybridization fragment pattern composition in comparison to Hays Golden. This population underwent irradiation with thermal neutrons before and after the first cycle of selection. Irradiation resulted in increased additive genetic variance, many aberrant plants, and reduced yield relative to Hays Golden (Gardner 1961, 1968). Selection among the limited number of relatively normal plants may have caused a founder effect for rDNA composition in the early generations that influenced later generation composition. It would be of interest to evaluate the earliest available generations of the irradiated population for possible founder effects in rDNA spacer composition. If there were no founder effects, the lack of overall change in the I-31 population would suggest that maize rDNA intergenic spacer composition is not always influenced by mass selection for yield, a complex trait.

Similar to C-29, the I-31 population demonstrated a significant increase in the frequency of the 5.2-kb fragment. This represents an increase in the frequency of chromosomes that have rDNA repeats without the SstI restriction site in the external transcribed spacer. Unless the base change responsible for loss of the restriction site confers some selective advantage, this fragment may function as a marker associated with loci under selection.

In addition, there were a few individual spacer hybridization fragment patterns in I-31 that demonstrated frequency changes similar to C-29, although of a smaller magnitude. These findings suggest that some limited selection of rDNA variants or associated loci may have occurred during the cycles of selection that generated the I-31 population. In view of the results in this paper, it would be worthwhile to examine whether rDNA intergenic spacer composition has changed in other plant breeding populations. This would further investigations on whether there is a selective advantage associated with some spacer hybridization fragment patterns. Establishment of such a relationship would then allow for the direct selection of certain spacer-length variants in plant breeding programs.

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