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Change in ribosomal DNA spacer-length composition in maize recurrent selection populations. 2. Analysis of BS10, BS11, RBS10, and RSSSC

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Abstract Four maize (*Zea mays* L.) populations selected for grain yield (BS10, Iowa Two-ear Synthetic; BS11, formerly Pioneer Two-ear Composite; RBS10, Illinois strain of BS10; and RSSSC, Illinois strain of Iowa Stiff Stalk Synthetic) were assayed for molecular variation in the ribosomal DNA (rDNA) intergenic spacer (IGS) at initial and advanced cycles of selection. RSSSC and RBS10 underwent reciprocal recurrent selection with an inbred tester in a high-yield environment, whereas BS10 and BS11 were subjected to full-sib reciprocal recurrent selection. Maize rDNA, which encodes the ribosomal RNA genes, is highly repetitive and shows IGS length variation within and among individuals. Five different ribosomal spacer-length variants (rslvs) and a polymorphic *Sst*I restriction site in the IGS were detected in the four populations. The five rslvs and the polymorphic restriction fragment were observed in 20 different combinations or hybridization fragment patterns (HP). RSSSC, RBS10, and BS11 showed significant changes in the overall rslv and HP frequencies between cycle 0 and the advanced cycle of selection, whereas BS10 did not. In general, two specific HPs were more frequent in the majority of the advanced cycles of the four populations. The frequency changes between initial and advanced cycles were more dramatic for HPs than rslvs. These results are consistent with earlier findings and further support the hypothesis that certain rDNA HPs and/or linked loci may be responding to selection for grain yield and may be associated with a selective advantage in US Corn Belt environments.

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Introduction

Eucaryotic ribosomal DNA (rDNA) is composed of an array of tandemly repeated units (Long and Dawid 1980; Jorgensen and Cluster 1988). Each unit is composed of the 18*s*, 5.8*s*, and 26*s* ribosomal RNA (rRNA) genes and the internal transcribed spacers. The whole unit is transcribed as a precursor rRNA that is then processed to form the mature subunits of the ribosome complex. Each rDNA transcription unit is separated from the adjacent repeat by a spacer that is located between the 3′ end of the 26*s* gene of one repeat and the 5′ terminus of the 18*s* gene of the next repeat. Only the spacer segment proximal to the 18*s* gene is usually transcribed as part of the rRNA precursor (Reeder 1989). Different nomenclature systems regarding the spacer can be found in the literature (Toloczyki and Feix 1986; Reeder 1989); we are using the term intergenic spacer (IGS) which is consistent with the terminology generally used for higher plants in recent years (Reeder 1989; Allard et al. 1990).

The size of a rDNA repeat unit in maize (*Zea mays* L.) is approximately 9.1 kb (Zimmer et al. 1988). The rDNA IGS in maize is approximately 3 kb long, and consists of two segments of different composition. One is a stretch of tandemly repeating sequences, the subrepeats, each about 200 nucleotides long (Fig. 1); and the other is a segment that contains transcription regulatory elements (McMullen et al. 1986; Toloczyki and Feix 1986). Maize rDNA repeats are concentrated on the short arm of chromosome VI, and different maize lines have been estimated to have between 5000 and 12000 copies of these repeats per haploid genome (Phillips et al. 1978).

The 18*s*, 5.8*s*, and 26*s* genes are highly conserved sequences, in contrast the IGS exhibits high interspecific and intraspecific variability. Within a species, the pri-

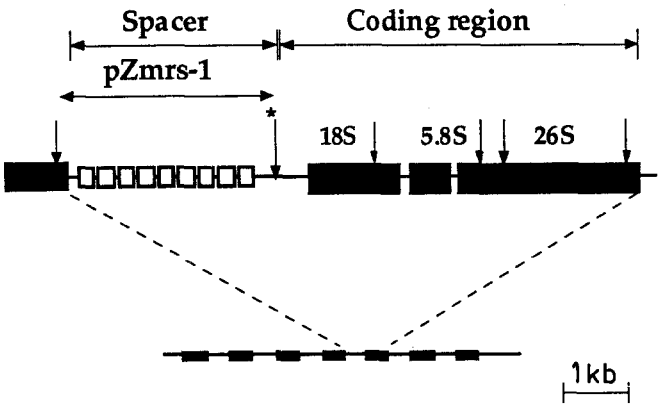


Fig. 1 Maize rDNA organization, an array of ribosomal genes (bottom), and an expanded view of a transcription unit. The average repeat unit is 9.1 kb, of which the spacer is approximately 3 kb (Zimmer et al. 1988). The *shaded boxes* represent sequences coding for the rRNA subunits. The spacer consists of a stretch of tandem subrepeats (*open boxes*), each about 200 nucleotides long, and a second segment that contains transcription regulatory elements (McMullen et al. 1986; Toloczyki and Feix 1986). The arrows indicate *Sst*I restriction sites, and the region of hybridization of the spacer probe pZmrs1

mary source of rDNA variation is the number of subrepeats within the IGS, this variation generates different ribosomal spacer length variants (rslvs). In maize, a series of five rslvs were found, varying in size from 3.4 to 4.2 kb in approximately 200-bp increments (Rocheford et al. 1990). Spacer-length variability was observed among different repeats of the ribosomal transcription unit within and among individuals. An additional source of variation is the different sets of rslvs on an individual chromosome homologue. A chromosome VI homologue can have all or some of the different rslvs; after fertilization, two different homologues create a pattern of variation that can vary considerably among individuals. These combinations are detected with a spacer-specific probe in a Southern-blot analysis as specific hybridization fragment patterns (Figs. 1, A) and are referred to as "hybridization patterns" (HPs).

Studies with different organisms have shown that the variation observed in the IGS may have functional and adaptive implications (Appels and Honeycutt 1986; Allard 1988; Reeder 1989). In *Xenopus laevis*, in vitro transcription experiments have shown that higher numbers of subrepeats in the IGS are associated with higher levels of rDNA transcription (Mitchelson and Moss 1987). In *Drosophila melanogaster*, a population selected for shorter developmental time exhibited a significant change in IGS composition with a shift toward longer rslvs (Cluster et al. 1987). An experimental population of wild barley (*Hordeum vulgare* ssp. *spontaneum*) generated by intercrossing 28 barley cultivars of world-wide origin underwent a directional change in the frequencies of rslvs and HPs after being grown under natural selection conditions in the Mediterranean climate of Davis, California. By generation 53 the predominant rDNA alleles (marked by different spacer-length variants) were the same as those found in the accessions adapted to the Mediterranean region (Saghai Maroof et al. 1984). Another explanation for the association between IGS variation and adaptation is that the IGS length variants serve as markers for different rDNA alleles of the coding regions of the ribosomal transcription unit that differ in their selective value (Allard et al. 1990). Alternatively, the different IGS variants or rDNA alleles may simply be linked to single-copy genes that respond to selection.

Maize is bred for adaptation to specific environments. If the ribosomal spacer has a selective significance, different spacer variants may be more advantageous in different environments. Selection may be associated with a directional change in the population spacer composition that will reflect these advantages. The effect of selection on the population structure for ribosomal IGS in maize was evaluated in two studies. In the first of these, two subpopulations of the open-pollinated variety Hays Golden selected for grain yield were analyzed (Rocheford et al. 1990). One subpopulation showed a significant change in rslv composition after selection, but the other did not. In the second study, two Iowa Stiff Stalk Synthetic populations, BS13 and BSSS(R), selected for combining ability by two different recurrent selection methods using different types of testers, were evaluated for rDNA IGS composition (Rocheford 1994). Significant changes in rslv and HP composition were observed with a different single HP becoming predominant in the advanced cycle of selection of each population. This suggests that different selection methods and testers influenced which HP became predominant in the Iowa Stiff Stalk Synthetic populations.

To explore further the changes in maize ribosomal IGS variation associated with different selection methods and selection in different environments, we assayed the population structure for the ribosomal spacer at early and advanced cycles of four maize populations: BS10, Iowa Two-ear Synthetic; BS11, formerly Pioneer Two-ear Composite; RBS10, Illinois strain of BS10; and RSSSC, Illinois strain of Iowa Stiff Stalk Synthetic. These populations represent different genetic backgrounds, different recurrent selection methods, different selection environments (Iowa versus Illinois), and two populations of common genetic background, since RBS10 is directly derived from cycle 4 of BS10.

Materials and methods

The populations

BS10, formerly named "Iowa Two-Ear Synthetic", and BS11, formerly "Pioneer Two Ear Composite", were subjected to full-sib reciprocal selection for yield (Hallauer 1967). RSSSC is a composite of four Stiff Stalk Synthetic populations: BSSS(R)C7, BSSSB(S_2)C1 and BSSS2(S_1)C2 (Kauffmann and Dudley 1979; Lambert 1984). RBS10 was developed from BS10 cycle 4 (C4) (Lambert 1984; Lu Hung-Shung 1986). RSSSC and RBS10 have undergone reciprocal recurrent selection with each other. Table 1 summarizes the origin and selection procedures used on the four populations.

Table 1 Summary of the origin and selection methodologies of the four maize populations evaluated for rDNA spacer variation

	BS10	BS11
Origin	Intermating ten lines with a strong two-ear expression.	Crossing southern prolific materials with US Corn Belt germ plasm
Selection scheme	Full-sib reciprocal selection	Full-sib reciprocal selection
Tester	BS11	BS10
Selected trait	Yield	Yield
	RSSSC	**RBS10**
Origin	A composite of four stiff-stalk synthetic populations	Initiated from BS10 cycle 4
Selection scheme	(1) Reciprocal recurrent selection (2) Phenotypic mass selection	(1) Reciprocal recurrent selection (2) Phenotypic mass selection
Tester	Inbreds derived from RBS10: B79(C1-C3), BS10-47(C3-C6)	Inbreds derived from RSSSC: B37(C1-C3), B84(C3-C6)
Selected trait	(1) Yield (2) Multiple disease resistance	(1) Yield (2) Multiple disease resistance

Sixty-nine individual plants were sampled from each of BS10 C0 and BS11 C0. Sixty-four individual plants from BS10 C10 and 78 individuals from BS11 C10 were sampled. RSSSC cycle 0 was represented by 64 individual plants and RBS10 C0 by 69 individual plants. Sixty-six individual plants from RSSSC C6 and 70 individual plants from RBS10 C6 were also sampled.

Southern blotting and hybridization

Lyophilized leaf tissue of individual plants was used for the DNA isolation procedure essentially as described by Saghai-Maroof et al. (1984). Three to five micrograms of DNA were digested with the restriction endonuclease *Sst*I (2.5 units per μg DNA) at 37 °C overnight; *Sst*I was selected because it generates a spacer-specific restriction fragment (Fig. 1). The use of *Sst*I in previous studies had revealed a restriction-site polymorphism at the 3′ end of the IGS. In those samples that lack this *Sst*I restriction site, the IGS plus the bulk of the 18*s* gene results in an additional 5.2-kb restriction fragment (Zimmer et al. 1986). The term "*Sst*I restriction-fragment length variants" will be used to describe the rslvs and the 5.2-kb fragment, and "hybridization pattern" (HP) will be used to describe the collective pattern of *Sst*I restriction-fragment length variants detected in a given plant DNA sample (Fig. 2 A).

DNA restriction fragments were separated by electrophoresis in 1% agarose gels. Transfer of DNA to nylon membranes and hybridizations were done according to standard procedures (Maniatis 1982). A 3.2-kb clone of the maize ribosomal spacer, pZmrs-1 (Fig. 1), produced by B. Hunter and provided by I. Rubenstein, Department of Genetics and Cell Biology, University of Minnesota, was used as a probe. On the resulting autoradiographs, bands that correspond to the different rslvs were scored as present or absent. Note that since the rslvs are not mutually exclusive, the sum of the rslv frequencies in a population do not add up to 1.

Statistical analysis

Chi-square tests for "goodness of fit" were used to compare rslv and HP frequencies between different cycles of selection of a population or between populations (Sokal and Rholf 1969). Shanon's information statistic was used to estimate the genetic variation of the populations (Bowman et al. 1971). Because of the overlapping nature of the HPs in some cases (Fig. 2 A), genotypic frequencies can not be calculated and, therefore, calculations of the relative contribution of random genetic drift to the observed changes in spacer variation were not performed.

Results and discussion

rDNA intergenic spacer hybridization fragment pattern frequencies

A total of 20 different hybridization fragment patterns (HPs) were observed among the four populations. None of the populations contained all 20, but rather they averaged 9.4 HPs per cycle of a population and ranged from 4 to 16 patterns per cycle. Ten of the observed HPs have not been reported among the 16 patterns described in Iowa Stiff Stalk Synthetic (BSSS) and in the maize variety Hays Golden (Rocheford et al. 1990; Rocheford 1994). Therefore, a total of 26 different HPs have been detected in maize thus far, indicating that extensive variation exists in this region of maize DNA.

Table 2 lists the different HPs observed in the four populations. Table 3 summarizes the frequency changes of the major HPs in the four populations and lists the results of a χ^2 comparison of their frequencies in the two cycles of selection. The major changes in each population are summarized below.

RSSSC

In cycle 0, 13 different HPs were observed. The most frequent HP, composed of 3.6/3.8/5.2-kb fragments, was present in 29.7% of the individuals (Table 2 A). After six cycles of recurrent selection, only four different HPs were detected. The 3.6/3.8/5.2 HP decreased to 14.75%, and the 3.6/3.8/4.2/5.2 pattern became more frequent. The 3.6/3.8/4.2/5.2 pattern increased from approximately 16% in cycle 0 to 82% in cycle 6, which was the largest increase observed in this study.

RBS10

Nine HPs were observed in RBS10 C0. Of these, three patterns accounted for 77% of the population (HPs

Table 2 The relative frequency (%) of hybridization patterns observed in RSSSC and RBS10 (A) and BS10 and BS11 (B) at the initial and advanced cycles of selection

A

Patterns	RSSSC CO	RSSSC C6	RBS10 C0	RBS10 C6
(1) 3.6	20.31	0	4.28	2.86
(2) 3.8	1.56	0	0	0
(3) 3.6/3.8	1.56	1.64	1.43	0
(4) 3.6/4.2	9.38	0	0	0
(5) 3.6/5.2	1.56	0	11.43	11.43
(6) 3.8/5.2	1.56	0	0	0
(7) 3.4/3.6/3.8	0	0	0	0
(8) 3.4/3.6/5.2	1.56	0	0	0
(9) 3.4/3.8/5.2	0	0	0	0
(10) 3.6/3.8/5.2	29.69	14.75	27.14	50
(11) 3.6/4.0/5.2	0	0	0	0
(12) 3.6/4.2/5.2	4.69	0	0	0
(13) 3.4/3.6/3.8/5.2	6.25	0	2.86	1.43
(14) 3.4/3.6/4.0/5.2	0	0	0	0
(15) 3.4/3.6/4.2/5.2	0	0	0	0
(16) 3.6/3.8/4.2/5.2	15.63	81.97	25.71	30
(17) 3.6/3.8/4.0/5.2	0	0	1.43	2.86
(18) 3.4/3.6/3.8/4.0/5.2	0	0	0	1.43
(19) 3.4/3.6/3.8/4.2/5.2	3.12	1.64	1.43	0
(20) 3.6/3.8/4.0/4.2/5.2	1.56	0	24.29	0

B

Patterns	BS10 C0	BS10 C10	BS11 C0	BS11 C10
(1) 3.6	1.47	8.19	7.25	2.53
(2) 3.8	0	0	0	0
(3) 3.6/3.8	1.47	3.28	0	1.27
(4) 3.6/4.2	0	0	0	0
(5) 3.6/5.2	23.53	21.31	26.64	12.66
(6) 3.8/5.2	0	0	0	0
(7) 3.4/3.6/3.8	0	1.64	0	3.8
(8) 3.4/3.6/5.2	5.88	0	4.35	7.6
(9) 3.4/3.8/5.2	0	0	0	1.27
(10) 3.6/3.8/5.2	32.35	42.62	26.24	10.13
(11) 3.6/4.0/5.2	0	0	0	3.8
(12) 3.6/4.2/5.2	1.47	0	1.45	2.53
(13) 3.4/3.6/3.8/5.2	19.11	19.67	13.04	10.13
(14) 3.4/3.6/4.0/5.2	0	0	0	1.27
(15) 3.4/3.6/4.2/5.2	0	0	0	1.27
(16) 3.6/3.8/4.2/5.2	7.35	1.64	14.49	22.78
(17) 3.6/3.8/4.0/5.2	0	0	0	0
(18) 3.4/3.6/3.8/4.0/5.2	0	0	0	0
(19) 3.4/3.6/3.8/4.2/5.2	5.88	10.14	10.14	13.92
(20) 3.6/3.8/4.0/4.2/5.2	1.47	0	0	3.8

number 10, 16, and 20 in Table 2 A). After six cycles of recurrent selection, three of the HPs observed in the initial cycle were not detected (HPs number 2, 19 and 20) and one new pattern (number 18 in Table 2 A) was observed, resulting in a total of seven HPs in cycle 6. Two patterns that were not detected in cycle 6 were in very low frequency in the initial cycle, but one pattern (number 20, Table 2 A) was one of the most abundant HPs (24.3% of the individuals) in cycle 0. HP 10 increased in frequency from 27.1% in cycle 0 to 50% in cycle 6. Therefore, in cycle 6, pattern 10 and pattern 16 comprised 80% of the population (Figure 2 B).

Table 3 χ^2 values and their probabilities from comparisons between the frequencies of the major hybridization patterns (relative frequency > 5%) in intial and advanced cycles of selection of the four maize populations (* = cells with $n < 5$ were pooled and tested together; – = the hybridization pattern was not observed in that population)

Patterns	RSSSC	RBS 10	BS10	BS11
3.6	12.39	*	*	2.43
	$P < 0.005$			NS
3.6/4.2	5.72	*	–	–
	$P < 0.025$			
3.6/5.2	*	0.33	0.128	5.8
		NS	NS	$P < 0.05$
3.4/3.6/5.2	*	*	*	*
3.6/3.8/5.2	4.58	13.45	1.99	8.13
	$P < 0.05$	$P < 0.001$	NS	$P < 0.01$
3.4/3.6/3.8/5.2	*	*	0.01	5.14
		NS	NS	$P < 0.05$
3.6/3.8/4.2/5.2	171.75	0.5	*	3.75
	$P < 0.001$	NS		NS
3.4/3.6/3.8/4.2/5.2	*	*	*	0.37
				NS
3.6/3.8/4.0/4.2/5.2	*	17	*	*
		$P < 0.001$		
* Pooled cells	6.75	0.5	3.095	8.97
	$P < 0.01$	NS	NS	$P < 0.01$
$\sum\chi^2$	201.19	31.28	5.22	34.59
	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$

RSSSC and RBS10

Comparisons of RSSSC and RBS10 in cycles 0 and 6 reveal that the populations were significantly different in their ribosomal HP composition at cycle 0 ($\chi^2 = 25.85$, $P < 0.005$) and, as is evident from the increase in the χ^2 value ($\chi^2 = 141.5$), they diverged further after selection. The inbred testers used in the reciprocal recurrent selection program of RSSSC and RBS10 were analyzed. The inbreds B79 and RBS10-47 were derived from BS10, and used to make testcrosses with RSSSC; B79 exhibited 3.6/3.8 and RBS10-47 exhibited 3.6/3.8/4.2/5.2 HPs. The inbreds B37 and B84, derived from Iowa Stiff Stalk Synthetic, were used as the testers for RBS10; B37 showed 3.6/3.8/4.0/4.2/5.2 and B84 showed 3.6/3.8/4.2/5.2 HPs. Each tester was used for three cycles (1–3 and 4–6) of recurrent selection. The testers were not random inbreds used to represent the source populations, but superior inbreds from the respective source population. It is noteworthy that the inbreds used for the last three cycles with both populations (BS10-47, B84) share the 3.6/3.8/4.2/5.2 HP, which is the same HP that became predominant in RSSSC C6 and was the second most frequent HP in RBS10 C

BS10

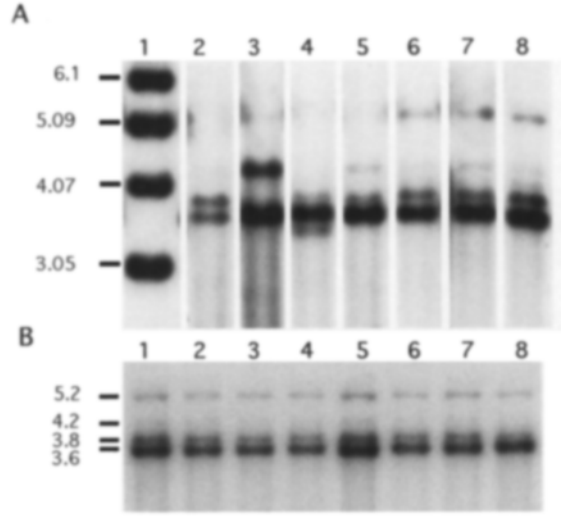
Ten different HPs were observed in BS10 C0. Three patterns (HPs 5, 10 and 13) comprised 75% of cycle 0 of the population (Table 2 B). After ten cycles of selection,

eight HPs were detected, and the same HPs were more frequent, accounting for 83.6% of the individuals in the population (Table 2B). The HP frequency changes in BS10 however, were not statistically significant.

BS11

The change in BS11 HP composition was different from the other three populations. In cycle 0, the two most frequent HPs (numbers 5 and 10, Table 2B) together accounted for 52.9% of the population, which is lower than the pooled frequencies of the most frequent HPs in the other populations. After ten cycles of selection, the two more frequent patterns in the initial cycle exhibited a significant decrease in frequency, and another pattern (number 17) became the most frequent (22.9%); the remaining 77.1% of the population was divided among 15 patterns of low frequency; eight of which were not detected in cycle 0 (Table 2B and Table 6). These eight HPs may be heterozygotes of two of the previously observed HPs (Fig. 2A), or alternatively are rare HPs and our cycle 0 sample size was too small to detect them. The general trend in BS11 was an increase in the number of different HPs, the majority of which were observed at a lower frequency.

Fig. 2A,B Different individuals can have all or some of the rslvs present in their genotype; this individual variation is detected upon hybridization to pZmrs1 as different hybridization patterns (HPs). A given HP can be heterozygous, the result of two chromosome-6 homologues that carry different rslv sets, or homozygous, the result of two identical chromosome-6 homologues. **A** Example of some of the HP variation present: *lane 2*, 3.6/3.8; *lane 3*, 3.6/4.2; *lane 4*, 3.4/3.6/3.8; *lane 5*, 3.6/3.8/4.2; *lane 6*, 3.6/3.8/5.2; *lane 7*, 3.6/3.8/4.2/5.2; *lane 8*, 3.6/3.8/4.0/4.2/5.2. In *lane 1*: a size marker (1-kb ladder) with the corresponding size designations in kilobases, (*lanes 2,5,7,8* from RSSSC C0, *lane 6* RSSSC C6, *lanes 3 and 4* from BS11 C0 and C10 respectively). **B** A representative part of RBS10 cycle-6 Southern blot demonstrating the reduction in genetic variation in terms of HPs, and the uniformity in the advanced cycle of selection



BS10 and BS11

Because BS10 and BS11 underwent reciprocal recurrent selection, the testcrosses between individual plants in the two populations are evaluated to identify superior full-sib families. Therefore, the two populations were compared for rDNA IGS composition. A comparison of BS10 and BS11, in cycle 0 revealed that the populations did not differ significantly in their ribosomal HP composition ($\chi^2 = 8.63$, $df = 4$, $P < 0.1$). A comparison of cycle 10 of BS10 and BS11, however, was associated with a highly significant χ^2 value (320.79, $P < 0.001$). This indicates that BS10 and BS11 diverged in their ribosomal HP compositions during ten cycles of reciprocal full-sib selection.

Ribosomal spacer-length-variant composition in the populations

Five different rslvs of approximately 3.4, 3.6, 3.8, 4.0, and 4.2 kb, and an additional 5.2-kb restriction fragment that is the result of a polymorphic *Sst*I restriction site, were found in populations RSSSC, RBS10, BS10 and BS11. These results are consistent with the rslvs and the *Sst*I-site polymorphism previously observed for maize rDNA (Zimmer et al. 1988; Rocheford et al. 1990; Rocheford 1994). The relative frequencies of the *Sst*I restriction-fragment-length variants in cycle 0 and the advanced cycle of selection of the four populations are listed in Table 4. RSSSC, RBS10, and BS11 showed an overall significant change in their rslvs and *Sst*I polymorphic-site frequencies, whereas frequencies within BS10 did not change (Table 5). No obvious pattern of

Table 4 Relative frequencies (%) of the *Sst*I restriction-fragment-length variants at initial and advanced cycles of selection of RBS10 and RSSSC(A) and BS10 and BS11(B) maize populations

A

Restriction fragments	RSSSC C0	RSSSC C6	RBS10 C0	RBS10 C6
5.2	71.88	100	94.2	97.14
4.2	37.5	75.76	52.17	30
4	1.56	3.03	26.09	4.29
3.8	60.94	100	84.06	85.71
3.6	96.88	100	98.55	100
3.4	7.81	0	4.35	2.86

B

Restriction fragments	BS10 C0	BS10 C10	BS11 C0	BS11 C10
5.2	94.2	89.06	88.41	93.59
4.2	13.04	3.12	26.09	46.15
4	1.45	0	0	10.26
3.8	65.22	65.63	62.32	66.67
3.6	100	96.88	100	100
3.4	30.43	20.31	27.54	41.03

Table 5 χ^2 values and their probabilities from comparisons between the frequencies of *Sst*I restriction length variants in two cycles of selection in the four maize populations

Restriction fragments	RSSSC	RBS10	BS10	BS11
5.2	7.26	0.06	0.18	0.24
	$P < 0.01$	NS	NS	NS
4.2	25.76	6.60	5.71	27.49
	$P < 0.001$	$P < 0.05$	NS	$P < 0.001$
4	–	12.75	–	–
	NS	$P < 0.001$	NS	NS
3.8	16.52	0.02	0.0	0.24
	$P < 0.001$	NS	NS	NS
3.6	0.45	0.0	0.06	0
	NS	NS	NS	NS
3.4	5.2	–	2.15	5.15
	$P < 0.05$	NS	NS	$P < 0.05$

change across the populations was observed, nor was there a particular rslv that was dominant in the populations. Noteworthy, however, was the uniformly high frequency of the 5.2-kb restriction fragment in the four populations (Table 4). The frequency of this fragment did not increase significantly in three of the four populations (Table 4), primarily due to the very high frequencies in the initial cycles. Nevertheless, the 5.2-kb fragment did not decrease in frequency, indicating that it may be associated with a favorable selection response.

The nature of change in population structure for rDNA spacer variants

Selection and random genetic drift are most likely responsible for the frequency changes observed in the ribosomal spacer. Unfortunately, the contribution of genetic drift in this study could not be estimated. Studies of different plant and animal systems have suggested that the genetic variation at the ribosomal spacer may be adaptive and that the region is under selection (see for example: Flavell et al. 1986; Cluster et al. 1987; Allard 1988). Assuming an adaptive value for maize ribosomal IGS, we would expect selection to result in a directional change in the relative frequency of different spacer genotypes. Directional change can be broken into two components: reduction in genetic variation, and convergence to the more adaptive variant.

Shannon's information statistic was used as a measure of the populations genetic variation at the initial and most advanced cycles of selection (Table 6). When variation is measured in terms of hybridization patterns, RSSSC, RBS10 and BS10 show reduction in their genetic diversity between cycle 0 and the advanced cycle (Fig. 2 B). This response is consistent with results previously obtained with maize populations BSSS(R) and BS13, and is characteristic of directional selection (Rocheford 1994). When the genetic variation is measured in terms of rslvs, only a slight decrease in the populations' ribosomal spacer variability in the advanced cycle was noted (Table 6). This suggests that the putative selective value of this region in maize may vary with the HPs rather than among the rslvs *per se*. A similar result was previously shown in barley in which the HPs differentiate cultivated barley from its wild relatives more distinctly than the rslvs (Saghai-Maroof et al. 1984). A recent inheritance study indicated that recombination is suppressed in the NOR region of maize (Simcox et al. 1995). Therefore, the HPs more accurately represent the actual hereditary unit of maize ribosomal genes than do the rslvs.

Table 6 Shannon's information statistic as a measure of the populations genetic diversity before and after selection, calculated from the *Sst*I restriction-fragment length variants data, and from the hybridization patterns data

Population Cycle	RSSSC		RBS10		BS10		BS11	
	C0	C6	C0	C6	C0	C10	C0	C10
*Sst*I restriction length variants	1.45	1.42	1.57	1.4	1.45	1.32	1.48	1.64
Hybridization patterns	2.04	0.58	1.71	1.28	1.8	1.53	1.87	2.37

The results for BS11, which showed an increase in genetic variation rather than a decrease between cycle 0 and cycle 10 (Table 6), are different from the other populations. The effect on the genetic variation in RSSSC, RBS10 and BS10 is characteristic of populations undergoing directional selection. In contrast, BS11 shows a pattern of change usually associated with relaxed selection. BS11 was developed from a more diverse germplasm than BS10, RBS10, and RSSSC, and contains germplasm from southern United States. BS11 has greater genetic variation than BS10 and RSSSC and the frequency for alleles commonly present in U.S. Corn Belt germplasm are probably at a lower frequency than for the other three populations. The different source of germplasm included in the formation of BS11 may explain in part the different HP frequencies compared with the other populations.

The predominant spacer variants after selection

The reduction in the genetic variability of the populations may be associated with a directional shift in the population structure toward better-adapted genotypes. These genotypes might be represented in our experiment as predominant HPs in the advanced cycle of selection. Table 7 lists the more frequent HPs before and after selection in the four populations. The results indicate that the populations underwent a differential increase in the relative frequency of some HPs at the expense of others during selection. In cycle 0 the most abundant HP in each of the four populations had frequencies in the range of 26.6 to 32.4%, and the differences between

Table 7 Number of rDNA IGS hybridization patterns observed in four maize populations, predominant hybridization patterns, and frequencies

Pop.	C0		Cadv	
	#Of patterns	Predominant hybridization pattern	#Of patterns	Predominant hybridization pattern
RSSSC	13	3.6,3.8,5.2 (29.7%) 3.6 (20.7%)	4	3.6,3.8,4.2,5.2 (82%) 3.6,3.8,5.2 (14.8%)
RBS10	9	3.6,3.8,5.2 (27.1%) 3.6,3.8,4.2,5.2 (25.7%)	7	3.6,3.8,5.2 (50%) 3.6,3.8,4.2,5.2 (30%)
BS10	10	3.6,3.8,5.2 (32.4%) 3.6,5.2 (23.5%)	8	3.6,3.8,5.2 (42.6%) 3.6,5.2 (21.3%)
BS11	8	3.6,5.2 (26.6) 3.6,3.8,5.2 (26.2%)	16	3.6,3.8,4.2,5.2 (22.8%) 3.4,3.6,3.8,4.2,5.2 (13.9%)

them and the next most frequent HP averaged 5.1% ± 4.2%. In the advanced cycle of selection, the most abundant HP accounted for 22.8 to 82% of the plants in the populations (Table 7), and the differences between them and the next most frequent HP increased on average to 32.8% ± 22.7%. In cycle 0, the predominant HP in RSSSC, RBS10, and BS10 was 3.6/3.8/5.2, which in BS11 was second highest in frequency to the 3.6/5.2 HP. In the advanced cycles of selection 3.6/3.8/5.2 was the most abundant HP in BS10 and RBS10, and 3.6/3.8/4.2/5.2 was the most abundant in RSSSC and BS11. Therefore, in the four experimental populations there was a directional shift toward two specific HPs (3.6/3.8/5.2 and 3.6/3.8/4.2/5.2).

Can we postulate an association between these two HPs with the selection of maize for yield in the U.S. Corn Belt environment? Such an association is conceivable in two ways: if the predominant HPs are linked to a favorable allele of a flanking gene, with a positive effect on yield or, alternatively, if the HPs vary in their selective value. The present study addresses the second option. Populations BS10 and RBS10 share the same genetic background (RBS10 cycle 0 = BS10 cycle 4), and underwent different selection schemes for grain yield in different environments (Table 1), yet the same HP became predominant (Table 7). In contrast, BS10 and BS11, which are diverse populations with similar ribosomal HP composition at cycle 0, were subjected to recurrent selection in the same environment with each other, but diverged in their ribosomal HP composition with different HPs becoming predominant (Table 7). These results suggest that the directional shift in the frequency of the HPs may not be solely a function of the ecological environment in which a population grows, but also a function of the populations genetic background and the genetic composition of the tester. Consequently, different maize populations may have a different favorable rDNA HP associated with selection in the U.S. Corn Belt.

The putative ribosomal spacer adaptiveness may be a complex function. rRNA transcription, is an interactive process that involves a number of elements. At present, three protein nuclear fractions that interact with IGS sequences, and are universally required for rRNA transcription, have been identified (Reeder 1992; Echeverria et al. 1994; Paule 1994). Therefore, the selective value of the ribosomal spacer may be a complex function of all the components involved in the regulation and transcription of rDNA. This function may change with the variation in each of its elements and the interplay between this genetic variation and the environment.

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