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Analysis of genetic recombination in maize populations using molecular markers *

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Summary. Variation in recombination rate is important to plant breeders since a major objective is to obtain favorable recombinants of linked genes. The ability to increase recombination (R) in circumstances in which favorable and unvavorable genes are linked (Corn Belt × exotic populations) and to decrease recombination when many favorable genes are linked (narrow-based, elite populations) would be of immense value. However, the concept of variation in recombination frequencies between linked genes has received limited attention despite its implications in breeding and genetic linkage studies. Molecular techniques have allowed better estimations of this variation. In this study, attempts were made to characterize: (1) the R values in the *Pgm1-Adh1* and *Adh1-Phi1* adjacent regions of chromosome 1 and the *Idh2-Mdh2* region of chromosome 6 in F_2 families of three maize (*Zea mays* L.) populations; (2) the environmental effect on R values of F_2s from two populations. One population, NSO, was a Corn Belt synthetic, and the other two populations, CBMEX3 and CBCAR5, were composites from crosses between Corn Belt and exotic germ-plams.

Wide ranges of estimated recombination ($\hat{R}$) values were observed among families in each population for all three chromsomal regions. The distribution of $\hat{R}$ values for the *Pgm1-Adh1* region showed that the F_2 families of each population fell into two broad categories: 0.30–0.50 and 0.02–0.20. No intermediates (0.21–0.29) were found. The distributions were almost normal for the *Adh1-Phi1* and the *Idh2-Mdh2* regions. It would appear that the major dispersion in the *Pgm1-Adh1* region was controlled by the effects of a single gene, while the *Adh1-Phi1* and *Idh2-Mdh2* regions were only affected by polygenes. No correlation was found between recombination values of the two adjacent regions, indicating that the genes affecting recombination for the *Pgm1-Adh1* region may be specific for that region.

For the *Pgm1-Adh1* region, no differences in $\hat{R}$ values were found among the three populations. For the *Adh1-Phi1* region, $\hat{R}$ frequencies of CBMEX3 and NSO were not significantly different, but both had significantly greater $\hat{R}$ values than CBCAR5. For the *Idh2-Mdh2* region, CBMEX3 was significantly different from NSO. There were significant differences between some paired F_2 families within each population for each chromosome region.

No significant differences in response to the two environments were detected in CBMEX3 and NSO for either region in chromosome 1.

Key words: Variation – Recombination frequency – Environmental effects – Maize

Introduction

Chromosomal recombination in plants is of significance in both naturally occurring and controlled breeding populations. Within natural populations it has been shown to evolve over time in such a manner that the population maintains a balance between fitness and flexibility for change by retaining adequate genetic variation (Dobzhansky et al. 1959; da Cunha and Dobzhansky 1954). In the conventional breeding program, the objective is to obtain favorable recombinants of linked genes. The fundamental factor controlling this process is the recombination (R) frequency between desirable and undesirable genes, or alternatively, the disruption of favorable linkage blocks. The ability to manipulate recombi-

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nation rates in either direction will certainly enhance progress from selection.

Previous studies on recombination frequencies had two basic limitations: (1) the use of chiasma frequency as a measure of recombination was a generalized approach and did not give information on variation in specific segments; (2) morphological markers were limited both in terms of variability and genetic background. Molecular markers, such as isozymes and restriction fragment length polymorphisms (RFLP's), have provided some distinct advantages in the study of R frequencies. The alleles at most of these loci are codominant which makes it possible to distinguish between heterozygotes and homozygotes. This saves time by avoiding test crosses and yields more statistical information in the computation of the estimated recombination ($\hat{R}$) value (Allard 1956). With rare exceptions, the allelic effects are not deleterious, in contrast to most morphological markers. Allelic variation is common at most loci in both broad-based and elite populations of maize. Thus, one can avoid the introduction of markers which may well disrupt the very phenomenon under study.

Variation in $\hat{R}$ frequencies of the same genes among individual lines of *Drosophila melanogaster* for different segments of the genome had been demonstrated as early as the beginning of this century (Sturtevant 1917; Goldschmidt 1917; Gowen 1919; Muller 1925). Subsequent studies in *Drosophila* have demonstrated the significant effect of inversions on crossing-over frequencies in the genome (Morgan et al. 1935; Levine and Dickinson 1952; Carson 1953; Levine and Levine 1954) and the heterotic effects in F_1s (Lawrence 1958). In maize, variability in crossing over in the adjacent regions *c-sh* and *sh-wx* of chromosome 9 was characterized by Stadler (1926). More recently, Beavis and Grant (1990) have reported that variation in R rates has somewhat hindered their attempts at constructing RFLP maps in maize.

Genotypic control of variation in crossing over was proposed, based on studies in rye that demonstrating signficantly less variation in chiasma frequency within lines than between lines and the presence of heterotic effects in F_1 individuals (Muntzing and Akdik 1948; Rees 1955, 1957; Rees and Thompson 1956). From the distribution of mean chiasma frequencies in inbreds, it was concluded that chiasma frequency was polygenically controlled (Rees 1955; Rees and Thompson 1956). The partitioning of the total variation in R frequencies into its components of genetic variance had shown that in *D. melanogaster* (Lawrence 1963) and *Hordeum* (Gale and Rees 1970) additive genetic variance accounted for all the measurable genetic variance, with very little dominance and no detectable epistatic effects.

Results of selection were inconsistent; some studies in *Drosophila* indicated no significant increases in R frequencies (Gowen 1919; Acton 1961), while others showed significant increases (Detlefsen and Roberts 1921; Parson 1958; Mukherjee 1961; Kale 1968; Dewies 1969). However, it seemed easier to select for decreased rates of R (Detlefsen and Roberts 1921; Detlefsen and Clemente 1923; Mukherjee 1961). The latter observation and the inconsistencies are understandable if we take into account the probable differences in genetic variability of the base populations. Additionally, the mating system used was inherently retrogressive in terms of preserving variability (Nei and Imaizumi 1968).

Single genes that modify the frequency of recombination have also been identified. Some of these were observed to affect frequency of chiasmata across the entire genome (Beadle 1930), while others tended to enhance or reduce R frequencies in specific segments of the genome (Enns and Larter 1962; Soost 1951; Lindsley et al. 1968; Moens 1969; Nel 1970a, b; Hinton 1970).

In addition to other extrinsic factors, climate, especially temperature, has a modifying effect. Studies in *Drosophila* (Plough 1917, 1921) and *Neurospora crassa* (Rifaat 1959; Towe and Stadler 1964; McNelly and Frost 1963) have established that the greatest effect of temperature was on chromosomal regions near the centromere. The optimum temperature range for increased R frequency varied with organism as well as the developmental stage of the organism. In *Drosophila*, increase were observed if the incubation temperature was increased from 22 °C to 31 °C or reduced to 13 °C (Plough 1917, 1921; Stern 1926; Graubard 1934; Lawrence 1963). Similar observations were made in plants in Liliaceae (Elliot 1955) and in barley (Powell and Nilan 1933). Rees (1957) demonstrated significant genotype by year interactions for chiasma frequency in rye lines.

The objectives of this study were: (1) to compare the estimates of recombination frequencies in two adjacent segments of chromosome 1 and another on chromosome 6 within and between three maize populations; and (2) to determine environmental effects on R in F_2 families of two of the populations.

Materials and methods

Plant materials

Three maize populations were used in this study; (1) CBMEX3 – a cycle-3 composite derived from crosses of Corn Belt and Mexican germ plasm; (2) CBCAR5 – a cycle-5 composite formed from crosses involving Corn Belt and Caribbean germ plasm; (3) NSO – a base population for breeding studies at Nebraska formed from crosses involving two improved Stiff Stalk Synthetic populations. The three populations were chosen since they allowed for comparisons between: (1) adapted versus adapted × exotic populations and (2) adapted × exotic versus adapted × exotic populations. Particular interest in the two exotic base populations was due to reports that Mexican and Caribbean germ plasm possess B chromosomes and knobs that have been shown to influence the overall frequency, as well as the distribution, of recombination. (Longley 1927, 1938; McClintock et al. 1981)

Table 1. Recombination ($\hat{R}$) values and SEs for the *Pgm1-Adh1* region of chromosome 1 in F_2 families of three maize populations (winter nursery, Fla., 1989)

CBMEX3			NSO			CBCAR5		
F_2 family	$\hat{R}$-value	n[a]	F_2 family	$\hat{R}$-value	n[a]	F_2 family	$\hat{R}$-value	n[a]
239	0.4999±0.05	100	364	0.4908±0.04	100	293	0.4725±0.07	50
251	0.4919±0.04	100	352	0.4870±0.04	102	282	0.4482±0.07	49
242	0.4904±0.04	100	363	0.4751±0.04	100	264	0.3908±0.06	50
231	0.4815±0.04	100	365	0.4361±0.04	100	267	0.2521±0.06	50
246	0.4453±0.04	100	373	0.4185±0.04	100	278	0.3454±0.05	56
232	0.4343±0.04	100	369	0.4125±0.06	50	285	0.3405±0.06	50
245	0.3892±0.04	99	371	0.3819±0.06	50	262	0.1165±0.03	50
241	0.3685±0.04	100	362	0.3710±0.06	50	272	0.0512±0.02	50
238	0.3306±0.04	99	366	0.1705±0.04	50	280	0.0202±0.01	50
233	0.3206±0.04	100	386	0.1584±0.02	100			
253	0.1158±0.03	50	354	0.1295±0.03	50		$\bar{R}$=0.2652±0.02	
256	0.1147±0.02	100	387	0.1269±0.02	100			
260	0.0944±0.03	50	355	0.1205±0.02	100			
259	0.0932±0.03	50	353	0.1161±0.03	50			
255	0.0898±0.02	99	397	0.0963±0.03	50			
252	0.0845±0.02	50	357	0.0878±0.02	100			
257	0.0727±0.02	50	394	0.0726±0.03	41			
234	0.0725±0.02	50	351	0.0620±0.02	51			
258	0.0723±0.02	50	391	0.0615±0.02	50			
236	0.0720±0.02	50	385	0.0513±0.02	50			
235	0.0620±0.02	50	356	0.0422±0.01	97			
249	0.0613±0.02	51	370	0.0409±0.02	50			
247	0.0517±0.02	50	390	0.0408±0.02	50			
244	0.0516±0.02	100	367	0.0305±0.01	50			
240	0.0412±0.20	50						
237	0.0409±0.02	50		$\bar{R}$=0.2093±0.01				
254	0.0305±0.01	50						
248	0.0254±≥0.01	100						
	$\bar{R}$[b]=0.2198±0.01							

[a] Number of individuals analyzed per family
[b] Estimated recombination value for the population using pooled F_2s

A random sample of seeds from each of the three populations was germinated, and coleoptilar tisses samples were taken. Seedlings were tagged and subsequently transplanted to the field, while the tissue was subjected to starch gel electrophoresis in order to identify genotypes of linked isozyme loci. Heterozygotes for linked loci were selfed because of inadequate variation in CBCAR5 and NSO. Contrasting double or triple homozygous individuals within each population were crossed to produce F_1 families, which were selfed to produce F_2 progenies. All F_1s were selfed in the winter nursery at Homestead, Florida, except for a duplicate set of F_1s from CBMEX3 and NSO, which were grown in the agronomy greenhouse, University of Nebraska-Lincoln. The objective was to determine the effect of environmental differences on R frequencies.

Fifty seedlings were first sampled for each F_2 family within each of the three populations. The numbers of F_2 families analyzed for CBMEX3, CBCAR5, and NSO were 28, 16, and 34, respectively.

Electrophoretic assays

Starch gel electrophoresis assays on crude extracts of coleoptile tissue based on the procedure of Brown and Allard (1969), Cardy et al. (1980), and Stuber et al. (1988), were used in this study. After electrophoresis the gels were sliced horizontally, stained for the respective enzyme, and read following the guidelines of Stuber et al. (1988).

Recombination value estimations and statistical analyses

Estimates of the recombination ($\hat{R}$) values were computed using the Linkage is computer program (Suiter et al. 1983) based on the maximum likelihood method of Allard (1956). Homogeneity of recombination values were tested by estimating genotypic frequencies based on the maximum likelihood estimates of the recombination values and using the likelihood ratio G^2 statistic (Bishop et al. 1975). Pearson chi-square tests were used to test the goodness-of-fit to a 1:2:1 ratio of segregating alleles at each locus in F_2 families. Individual tests of segregation distortion used adjusted comparison-wise error rates to keep the per-experiment error rate less than or equal to 0.05.

Results

F_2 recombination values for Pgm1-Adh1 and Adh1-Phi1 chromosome regions from Florida winter nursery material

The number of F_2 families available for R analysis in each population depended upon the allelic variability.

Table 2. Recombination ($\hat{R}$) values and SEs for the *Adh1-Phi1* region of chromosome 1 in F_2 families of three maize populations (winter nursery, Fla., 1989)

CBMEX3			NSO			CBCAR5		
F_2 family	$\hat{R}$-value	n[a]	F_2 family	$\hat{R}$-value	n[a]	F_2 family	$\hat{R}$-value	n[a]
257	0.2413±0.05	50	390	0.2340±0.04	50	282	0.2161±0.05	50
249	0.2342±0.04	50	356	0.2294±0.03	100	284	0.1542±0.03	50
260	0.2068±0.04	50	391	0.1936±0.04	49	272	0.1380±0.03	50
244	0.2000±0.03	100	372	0.1926±0.04	50	267	0.1191±0.03	50
238	0.1740±0.03	100	354	0.1899±0.04	50	285	0.1058±0.03	50
256	0.1720±0.03	100	378	0.1898±0.04	60	262	0.1058±0.03	50
241	0.1606±0.02	100	392	0.1804±0.04	50	268	0.0954±0.03	50
240	0.1558±0.04	50	352	0.1753±0.03	100	290	0.0845±0.02	50
246	0.1514±0.02	100	365	0.1746±0.03	100	269	0.0839±0.02	50
236	0.1476±0.03	50	366	0.1726±0.04	50	278	0.0835±0.02	50
235	0.1301±0.03	49	374	0.1693±0.04	49	264	0.0830±0.02	50
231	0.1280±0.02	99	357	0.1455±0.03	90	270	0.0731±0.02	52
234	0.1173±0.03	50	370	0.1534±0.03	50	276	0.0622±0.02	50
259	0.1162±0.03	50	368	0.1510±0.03	50	283	0.0622±0.02	50
235	0.1161±0.03	49	363	0.1410±0.02	100	293	0.0513±0.02	50
239	0.1112±0.02	100	355	0.1272±0.03	101	280	0.0305±0.01	50
232	0.1105±0.02	96	369	0.1285±0.03	50			
251	0.1065±0.02	99	375	0.1271±0.03	50	$\bar{R}=0.0956\pm0.01$		
242	0.1061±0.02	100	362	0.1257±0.03	50			
248	0.1054±0.02	100	376	0.1257±0.03	50			
237	0.1046±0.03	50	394	0.1178±0.03	50			
233	0.0969±0.02	99	373	0.1167±0.02	100			
255	0.0902±0.02	99	359	0.1161±0.02	50			
247	0.0841±0.02	100	358	0.1111±0.03	50			
258	0.0836±0.02	50	397	0.1062±0.03	50			
254	0.0828±0.02	50	353	0.0959±0.03	50			
245	0.0727±0.01	100	351	0.0951±0.03	50			
252	0.0518±0.02	50	385	0.0951±0.03	50			
			367	0.0944±0.03	50			
$\bar{R}$[b] $=0.1276\pm0.01$			361	0.0835±0.02	50			
			364	0.0731±0.01	99			
			371	0.0618±0.02	50			
			387	0.0615±0.01	100			
			386	0.0461±0.01	100			
			$\bar{R}=0.1322\pm0.01$					

[a] Number of individuals analyzed per family
[b] Estimated recombination value for the population using pooled F_2s

Table 1 contains $\hat{R}$ values for 28, 24, and 9 F_2 families from CBMEX3, NSO, and CBCAR5, respectively, for the *Pgm1-Adh1* segment. This region showed a wide range of $\hat{R}$ values (0.02–0.50) in all three populations. The distribution (Table 3) showed a gap in the middle from 0.20 to 0.30.

Recombination values for an adjacent chromosome region, *Adh1-Phi1*, are reported in Table 2. The numbers of families used were 28, 34, and 16 for the three populations. Frequencies, shown in Table 3, reveal that the range of $\hat{R}$ values is only about one-half that in the other region, ranging from 0.05 to 0.24. The family values of this set appeared to be more nearly normally distributed.

Recombination values differed significantly ($P<0.001$) among populations for both the *Pgm1-Adh1* region (0.22, 0.21, and 0.27) (Table 1) and the *Adh1-Phi1* region (0.13, 0.13, and 0.10) (Table 2). Tests of homogeneity of $\hat{R}$ values among F_2 families within populations indicated significant heterogeneity ($P<0.03$) among families in all populations for each of the two chromosomal regions.

Segregation ratios of the marker loci, *Pgm1* and *Adh1*, for region 1 of chromosome 1 in CBMEX3 and NSO were computed in order to determine deviations from a 1:2:1 segregation and are reported in Table 4. In both populations the number of F_2 families demonstrating distorted ratios was higher for the *Pgm1* locus than for the *Adh1* locus. The F_2 families are depicted in two categories. In category (i), F_2s had $\hat{R}$ values ranging from 0.3 to 0.5. In category (ii) the $\hat{R}$ values were between 0.02

Table 3. Frequencies of F_2 families from three populations in five classes of recombination rates for two chromosome segments (winter nursery, 1989); calculated from Tables 1 and 2

Frequency class	CBMEX3	NSO	CBCAR5
	Pgm1-Adh1 (From Table 1)		
	28[a]	24[a]	9[a]
0.01–0.099	0.57	0.42	0.22
0.10–0.199	0.07	0.25	0.11
0.20–0.299	0	0	0
0.30–0.399	0.14	0.08	0.44
0.40–0.499	0.21	0.25	0.22
	Adh1-Phil (From Table 2)		
	28[a]	34[a]	16[a]
0.01–0.049	0	0.03	0.06
0.05–0.099	0.25	0.24	0.56
0.10–0.149	0.43	0.32	0.25
0.15–0.199	0.18	0.35	0.06
0.20–0.249	0.14	0.06	0.06

[a] Number of F_2 families analyzed in each population

Table 4. Chi-square analysis of segregations (compared to a 1:2:1 ratio) of marker loci for the *Pm1-Adh1* region of chromosome 1 (winter nursery, Fla., 1989)

Population	Category[a]	Locus	F_2 families	
			Total number	Number w/significant** distorted ratio
CBMEX3	(i)	*Pgm1*	10	1
	(i)	*Adh1*	10	0
	(ii)	*Pgm1*	18	0
	(ii)	*Adh1*	18	0
NSO	(i)	*Pgm1*	8	0
	(i)	*Adh1*	8	0
	(ii)	*Pgm1*	16	2
	(ii)	*Adh1*	16	0

** Significant at the 0.05/(number of families) probability level – used to keep the per-experiment error rate less than or equal to 0.05 for this category

[a] (i), F_2s with $\hat{R}$-value 0.30–0.49; (ii), F_2s with $\hat{R}$-value 0.02–0.20

Table 5. Recombination ($\hat{R}$) values and SEs for the Idh_2-Mdh_2 region of chromosome 6 in S_1 families of three maize populations

Greenhouse			Field					
CBMEX3			NSO			CBCAR5		
Family	$\hat{R}$-value	n[a]	Family	$\hat{R}$-value	n[a]	Family	$\hat{R}$-value	n[a]
2	0.1009±0.02	62	7	0.0653±0.02	71	13	0.0487±0.01	63
3	0.1682±0.03	74	13	0.0410±0.02	50	17	0.0995±0.02	74
7	0.1383±0.01	74	14	0.0484±0.02	46			
10	0.0485±0.01	114	16	0.0672±0.02	47			
19	0.1059±0.02	89	18	0.0216±0.01	42			

[a] Number of individuals analyzed per family

and 0.20. For CBMex3 and *Pgm1*, category (i) had a higher frequency of distorted ratios than (ii). For NSO and *Pgm1*, category (ii) had a higher number of F_2s with distorted ratios.

Recombination values for the Idh2-Mdh2 region of chromosome 6 from S_1 families, Lincoln

Recombination values can also be obtained from segregating progenies of selfed plants (S_1s) heterozygous for alleles at linked loci (Ritter et al. 1990). This was done for the *Idh2-Mdh2* region of chromosome 6 (Table 5). In spite of the small number of S_1s analyzed for each population, the $\hat{R}$ value range for CBMEX3 (0.05–0.17) was greater than that of families of NSO (0.02–0.07). In the case of CBCAR5 only two families were analyzed.

Effect of environment on recombination frequencies

The two environments tested using CBMEx3 and NSO F_2 families were the winter nursery, Homestead, Florida and the agronomy greenhouse, Lincoln, Nebraska. Our primary interest was to determine whether populations responded differently in the two environments respect to $\hat{R}$ values for the two regions of chromosome 1. Likelihood ratio G^2 tests revealed no significant ($P<0.05$) location or population × location interaction for the *Pgm1-Adh1* region and no location or population or population × location interaction for the *Adh1-Phi1* region. Table 6 illustrates recombination values for CBMEX3 and the two locations for both chromosomal regions.

Table 6. Recombinant values for two regions of chromosome 1 in F_2 families of CBMEX3 at two locations – winter nursery, Fla. and greenhouse, Lincoln, Neb.

F_2 family	Recombination values			
	Greenhouse	n[a]	Winter nursery	n[a]
(1) CBMEX3 – *Pgm1-Adh1* region				
231	0.4808±0.04	100	0.4815±0.04	100
232	0.4162±0.04	100	0.4343±0.04	100
244	0.0356±0.01	100	0.0516±0.02	100
245	0.3568±0.04	100	0.3892±0.04	99
255	0.0460±0.01	100	0.0898±0.02	99
256	0.0510±0.01	100	0.1147±0.02	100
	$\hat{R}$[b] = 0.2154±0.01		$\hat{R}$ = 0.2506±0.01	
(2) CBMEX – *Adh1-Phi1* region				
231	0.1168±0.02	101	0.1280±0.02	99
232	0.1838±0.03	100	0.1105±0.02	96
244	0.1943±0.03	100	0.2000±0.03	51
245	0.1219±0.02	100	0.0727±0.01	100
255	0.1218±0.02	100	0.0902±0.02	99
256	0.1372±0.02	100	0.1720±0.02	100
	$\hat{R}$ = 0.1456±0.01		$\hat{R}$ = 0.1284±0.01	

[a] Number of individuals analyzed per family
[b] Estimated recombination value for the location

Discussion

Wide variation in recombination frequencies for specific chromosomal regions among random F_2 families from three maize populations was documented in this study. Both the *Pgm1-Adh1* and the *Adh1-Phi1* regions had apparently continuous variation between 0.01 and 0.20 frequencies, and the latter had some values between 0.20 and 0.25 (see Table 2). The *Pgm1-Adh1* region showed no frequencies between 0.20 and 0.30 and then had another apparently continuously variable set of values ranging from 0.30 to 0.50.

Genetic control in the two regions could have similar underlying mechanisms in that both show what appears to be continuous variation, presumably resulting from several to many loci, each having small effects. However, the discontinuity for the *Pgm1-Adh1* region suggests a single major gene effect. The ratios of the low-to-high rates in the three populations are 64/35, 67/33, and 33/66. If two alleles were involved, and the one controlling the low rate was dominant to the other, the dominant alleles frequency could have been lower than the recessive, so that the homozygous dominant plus the heterozygote would be about 2 times the frequency of the homozygous recessive for the first two populations above. For example, if the frequency of allele A is 0.4 and that of *a* is 0.6, the genotypic frequency of *AA* plus *Aa* genotypes (0.16 + 0.48 = 0.64) is about twice the frequency of the *aa* genotype (0.36) following the Hardy-Weinberg distribution. The ratio in the other population could be achieved if the frequency of *A* was 0.2. There are also other possibilities, e.g., the frequency of *a* could be 0.8, with *a* controlling the low rate in the first two populations above. An experiment to determine the best genetic explanation would be to cross families with $\hat{R}$ values between 0.01 and 0.20 in the *Pgm1-Adh1* region with those between 0.30 and 0.50. After selfing to form F_2 families in each cross, segregation ratio can then be observed to allow one to choose among alternative genetic explanations. This work is now underway.

Rees (1955) also concluded that control of R frequency was polygenic, based on distributions of mean chiasma frequencies using rye inbreds. Furthermore, Lawrence (1963) and Gale and Rees (1970) in studies on *Drosophila* and *Hordeum*, respectively, determined that gene effects controlling chiasma frequency are primarily additive. In our study, the discontinuity observed in the *Pgm1-Adh1* region definitely suggests that a single locus with major effects is involved in that segment. The monogenic control of R frequency in various segments of the maize genome has been previously reported (Beadle 1930; Nel 1970a).

The challenge from this work is not the fact that variation in R rates exist, because this had been demonstrated previously, expecially in other species, but that the variation is so great. Certainly these observations suggest questions about the meaningfulness of genetic maps that are based on recombination frequencies. Beavis and Grant (1991) observed "detection of unequal recombination among populations raises the question of whether the data should be pooled and a composite map constructed". Perhaps the ordering of loci along the chromosome is the best function that recombination map making can serve.

The occurrence of distorted segregation ratios for the *Pgm1* and *Adh1* loci did not indicate that a particular population (across loci) or locus (across populations) had a significantly higher proportion of F_2 progenies with distorted ratios. This would indicate that the distorted ratios did not contribute to the observed recombination frequencies. The exact mechanism favoring distorted ratios of marker loci was not evident in this study. In a study where sample size for two F_2 maize families were 1930 and 1976 individuals, respectively, Edwards et al. (1987) found a good fit for all 17 marker loci in one family, but in the other F_2, 12 out of 20 marker loci exhibited distorted ratios. Deviations of segregating loci from the expected ratio are commonly encountered in genetic linkage map construction (Slocum et al. 1990), but are not always reported. The general postulate was that the mechanism must have occurred prior to zygote

development, since ears were full and kernel germination was normal.

No significant correlation were found between $\hat{R}$ frequencies of the adjacent regions *Pgm1-Adh1* and *Adh1-Phi1* on chromosome 1. Thus the factor controlling very high recombination frequency in the *Pgm1-Adh1* region did not cause very high recombination in the *Adh1-Phi1* region. Nel (1970a) described a similar situation in maize, where a recessive gene that resulted in increased recombination in the *A2-Bt1* region of Chromosome 5 had no effect in the *Bt1-P2* adjacent region.

The distribution of $\hat{R}$ values between populations for the *Idh2-Mdh2* region of chromosome 6, as seen in Table 5, seemed to follow that of the *Adh1-Phi1* regions, though this interpretation should be considered tentative due to the small number of families examined in each population. At least, no very high recombination rates were observed for that region.

Most studies have reported a positive effect of temperature on $\hat{R}$ frequencies. However, this effect is related to the proximity of the tested chromosome segment to the centromere. Mather (1939) proposed that the greater sensitivity of proximal chromosomal regions was modulated by centromeric hetrochromatin. The regions measured in this study are located on the distal half of the long arm of chromosome 1. Some studies, however, have not detected significant enhancement of recombination frequency in proximal regions (White 1934; Lawrence 1963). Other studies (Powell and Nilan 1963) have shown the effect of temperature to be specific for regime (temperature) as well as development stage. It would appear, therefore, that the effect of enviroment on recombination is quite complex. More comprehensive research is required to separate environmental effects from genetic effects in R rate variation.

Although heterochromatin can influence R rates, such effects were not likely to have been a major factor in this study. B chromosomes and abnormal 10 could have been involved in families from CBMEX3 and CBCAR5, but have not been found in Stiff Stalk Synthetic from which NSO was derived. With the limited numbers of cytological observations made (five plants in CBMEX3), no evidence of abnormal 10 was found, but one plant with a pair of B chromosomes was found.

A long-term goal of this study was to recombine families exhibiting high and low $\hat{R}$ rates to produce populations with divergent recombination rates. This has now been done, and the tests of rate divergency will be done in the near future.

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