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Evidence for multilocus genetic control of preferential fertilisation in maize

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Genetic segregation was studied in more than 1900 seedlings of an F_2 between the maize (*Zea mays* L.) inbred lines T232 and CM37. Significant segregation distortion was observed at 11 of 17 segregating allozyme loci and at a single morphological marker locus distributed on 7 of the 10 chromosomes in the genome. Deviations from genotypic class expectations were small for most loci, and averaged 7·7 per cent. Percent transmission of the allele contributed by T232 varied from 47·7 per cent to 53·3 per cent. The allele donated by T232 was significantly under-represented for loci on chromosomes 1 and 8, whereas the allele contributed by CM37 was deficient for nine of the ten segregating loci on chromosomes 2, 3, and 6. In all cases, the parental origin of the deficient allele was consistent for markers on a chromosome. Evidence is presented that suggests the aberrant ratios arose from linkage of the markers with genetic factors affecting prezygotic transmission, and that a minimum of 5 such factors were operative, one on each of chromosomes 1, 2, 3, 6, and 8. In contrast to the multi-locus and multi-chromosomal distorted segregation observed in the F_2, all loci in backcross progenies fit Mendelian expectations. It is suggested that this discrepancy reflects variable environmental selection pressures on genes that influence aspects of gamete competition.

INTRODUCTION

One of the fundamental tenets of classical Mendelian genetics is that segregation of alternate alleles in a heterozygous individual occurs randomly during gametogenesis, and that the progeny array resulting from the fusion of randomly transmitted gametes is predictable. There are, however, numerous examples of non-random transmission in plants, which are usually detected genetically as distorted segregation of one or more genes in particular progenies (see Grant, 1975). Aberrant segregation ratios in plants may arise from a variety of physiological or genetic causes, and may be manifested as differential transmission in either the male (Bianchi and Lorenzoni, 1975; Mangelsdorf and Jones, 1926; Pfahler, 1975) or female (Longley, 1945; Rhoades, 1952; Rhoades and Dempsey, 1966) germ line, or result from post-zygotic selection prior to genotypic censusing (*e.g.* Rick, 1963). Most commonly, however, skewed segregation appears to arise from male gametophytic selection, through selective influences of the gynoecium (Johnson and Mulcahy, 1978; Ottaviano *et al.*, 1982; Pfahler, 1967), the environment (Zamir *et al.*, 1982), or through differential competitive ability of genetically variable pollen (Mangelsdorf and Jones, 1926; Pfahler, 1975; Sprague, 1933). In most cases, the aberrant ratios are restricted to one or a few marker genes that are hypothesised to be linked with unobserved genetic factors influencing some aspect of gamete function (Emerson, 1934; Mangelsdorf and Jones, 1926). The possibility that numerous factors may influence male competitive ability has recently been suggested by selection experiments in maize (Mulcahy, 1974; Ottaviano *et al.*, 1982).

In the course of investigating the genetic control of quantitative trait variation in maize (Edwards *et al.*, 1986; Stuber *et al.*, 1986), an unusual F_2 progeny arose where skewed segregation ratios were observed for a large proportion of the molecular markers scored. These data are presented here, along with evidence that suggests the aberrant ratios arose through multi-locus and multi-chromosomal gamete competition.

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MATERIALS AND METHODS

During the summer of 1983, F_1 hybrids between the two inbred lines CM37 and T232 were produced in a field near Clayton, N.C. These lines are divergent in pedigree, morphology, and allozyme genotypes: CM37 is an early-maturing Canadian dent whereas T232 was derived from an admixture of the open-pollinated varieties Jellicorse (a Southern Dent) and Teko Yellow (from South Africa). The two lines are homozygous for different alleles at 17 allozyme loci (Stuber and Goodman, 1983*a*; see table 1). Subsequent to harvest, the hybrids were electrophoretically verified, grown in a winter nursery near Homestead, Fla., and selfed to produce the F_2 generation. Backcross progenies with T232 were generated in the summer of 1985 in North Carolina, using the F_1 as both male and female. All families were produced in the cytoplasm contributed by T232.

Starch gel electrophoretic analyses were conducted on etiolated coleoptile tissue of 5-day-old seedlings following established procedures (Cardy *et al.*, 1983; Goodman *et al.*, 1980; Stuber and Goodman, 1983*a*, 1983*b*). For the analysis of the F_2 progeny, over 1900 seedlings were scored for their genotypes at 17 segregating allozyme loci, and subsequent to transplanting to the field, were additionally scored for the color factor *B* (Booster). Segregating marker loci were present on seven of the ten maize chromosomes. Parental genotypes, locus symbols, and chromosomal locations of most allozyme loci have been previously detailed (Goodman and Stuber, 1983; Stuber and Goodman 1983*a*, 1983*b*; Wendel *et al.*, 1985, 1986), and are reproduced in table 1. Electrophoretic methods and variation patterns for two of the 17 segregating allozyme loci (*Tpi*-4 and *Dia*-1) are unpublished. Formal genetic documentation of these markers will be presented in a future communication. Backcross progenies were similarly analysed, although the numbers were smaller (N = 202 and 406 for $F_1 \times T232$ and $T232 \times F_1$, respectively) and the seedlings were not transplanted.

Most loci segregated three genotypic classes in the F_2 and two classes in the BC generations. Exceptions are at Booster, where the allele *B* is dominant to *b*, and at the allozyme loci *Mdh*-1, *Mdh*-5, *Hex*-1, and *Tpi*-4, where only two of the three genotypic classes could be discriminated. These cases are due either to the presence of null variants in one parent (in which case the null/active heterozygous class was pooled with the active homozygous class), or to band overlap with the products of other loci. Booster (*B*) and *Hex*-1 were not scored in the BC progenies.

Segregation at each locus was tested for conformance to Mendelian expectations by chi-square goodness of fit tests. Recombination estimates among linked loci were calculated according to the method of maximum likelihood using the computer program Linkage-1 (Suiter *et al.*, 1983).

Table 1 Chromosomal locations* and parental genotypes of allozyme loci differentiating the maize inbred lines T232 and CM37

Enzyme	Locus symbol	Chromosomal location	T232 allele	CM37 allele
Acid phosphatase	*Acp*-1	9-60	4	2
Alcohol dehydrogenase	*Adh*-1	1L-127	4	6
Aminopeptidase	*Amp*-3	5S-32	3	4
Aspartate aminotransferase	*Got*-2	5L-96	4	2
Diaphorase	*Dia*-1	2S-27	12	8
Esterase	*E*-8	3S-20	4	4.5
Hexokinase	*Hex*-1	3S-44	4	*n*
	Hex-2	6L-58	2	1
Isocitrate dehydrogenase	*Idh*-2	6L-101	4	6
Malate dehydrogenase	*Mdh*-1	8-0	1	6
	Mdh-2	6L-103	6	3.5
	Mdh-3	3L-121	18	16
	Mdh-5	5S-16	15	12
Phosphoglucomutase	*Pgm*-2	5S-0	4	3
6-Phosphogluconate dehydrogenase	*Pgd*-2	3L-71	2.8	5
Phosphohexose isomerase	*Phi*-1	1L-139	4	5
Triose phosphate isomerase	*Tpi*-4	3S-61	*n*	4

* Approximate map positions are indicated, where L and S refer to the long and short arms, respectively. *Acp*-1 and *Mdh*-1 are near the centromeres of their respective chromosomes (arm not known). Recombination distance (in cM) between any two markers on a chromosome is equal to the difference in their map positions; *viz*, *Tpi*-4 (3S-61) and *Pgd*-2 (3S-71) recombine with a frequency of 10 per cent.

RESULTS AND DISCUSSION

Recombination estimates between all pairs of linked loci in the F_2 and BC families were calculated and compared to previously published data (Goodman *et al.*, 1980; Goodman and Stuber, 1983; Wendel *et al.*, 1985, 1986). All linkage estimates were statistically homogeneous (data not presented), indicating no significant recombinational differences among the families.

Segregation data for all loci in the F_2 and backcross generations are presented in table 2. In the F_2 progeny, observed segregations for all but six loci significantly departed from expectations ($p < 0{\cdot}10$). Twelve loci showed aberrant segregation, three at the 10 per cent level, five at the 5 per cent level, and four at the 1 per cent level. The observed magnitude of distortion was generally small, however; the average deviation from genotypic class expectations was 7·7 per cent, and transmission of the T232 allele varied from 47·7 to 53·3 per cent. The ability to detect significant deviations from expectations for some loci was clearly due to the large sample sizes employed. It is likely that with smaller sample sizes fewer cases of distortion would have been observed.

Of particular interest are the chromosomal locations of the markers that displayed skewed segregation, and the parental origins of the deficient alleles. Deviant ratios were observed for nearly all loci on five of the seven marked chromosomes: both segregating loci on chromosomes 1 and 2, four of five markers on chromosome 3, all three markers on chromosome 6, and the single segregating locus on chromosome 8. In contrast, all four markers on chromosome 5 fit Mendelian expectations, as did the single marker on chromosome 9. Furthermore, the deficient parental class varied among the chromosomes, but was consistent for multiple markers on any given chromosome. The allele donated by T232 was significantly underrepresented for markers on chromosomes 1 and 8,

Table 2 Segregation* of genetic markers in F_2's and backcrosses among the maize inbred lines T232 and CM37

	F_2					F_1 as female				F_1 as male			
Locus	T/T	T/C	C/C	%	χ^2	T/T	T/C	%	χ^2	T/T	T/C	%	χ^2
Chromosome 1:													
Adh-1	438	987	503	48·3	5·48*	100	102	49·5	0·02	161	188	46·1	2·09
Phi-1	435	969	524	47·7	8·27**	96	106	47·5	0·50	169	189	47·2	1·12
Chromosome 2:													
Dia-1	518	967	440	52·0	6·36**	85	100	45·9	1·22	138	127	52·1	0·46
B	504	1336	—	—	5·61**	—	—	—	—	—	—	—	—
Chromosome 3:													
E-8	463	971	494	49·2	1·10	95	107	47·0	0·71	199	207	49·0	0·16
Hex-1	—	1448	437	—	5·42**	—	—	—	—	—	—	—	—
Tpi-4	556	1372	—	—	15·15**	99	103	49·0	0·08	164	142	53.6	1·58
Pgd-2	574	908	446	53·3	23·50***	95	105	47·5	0·50	210	194	52·0	0·63
Mdh-3	515	971	442	51·9	5·63*	104	98	51·5	0·18	215	191	53·0	1·42
Chromosome 5:													
Pgm-2	489	951	488	50·0	0·35	97	102	48·7	0·13	182	208	46·7	1·73
Amp-3	512	933	480	50·8	2·87	90	112	44·6	2·40	212	194	52·2	0·80
Mdh-5	—	1430	498	—	0·71	96	106	47.5	0·50	202	194	51·0	0·16
Got-2	489	951	488	50·0	0·35	95	107	47·0	0·71	180	176	50·6	0·04
Chromosome 6:													
Hex-2	488	1021	416	51·9	12·50***	94	107	46·8	0·84	209	194	51·9	0·56
Idh-2	523	953	451	51·9	5·61*	104	98	51·5	0·18	210	195	51·9	0·56
Mdh-2	525	956	447	52·0	6·44**	105	97	52·0	0·32	207	195	51·5	0·36
Chromosome 8:													
Mdh-1	—	1320	583	—	32·24***	95	107	47·0	0·32	188	218	46·3	2·22
Chromosome 9:													
Acp-1	499	924	487	50·3	2·16	92	109	45·8	1·78	174	168	50·9	0·11

* $N = 1928$, 202, and 402 for the F_2, BC with F_1 as female, and BC with F_1 as male families, respectively, although some loci in some individuals were not scored. The designations T/T, T/C, and C/C refer to homozygosity for the T232 allele, heterozygosity, and homozygosity for the CM37 allele, respectively. Percent T232 representation in the progenies (where calculable) is also indicated (%). Expected ratios were 1:1 in the BC's and 1:2:1 in the F_2, except for the loci B, *Hex*-1, *Tpi*-4, *Mdh*-5, and *Mdh*-1, where the expected ratio was 3:1. Significance levels for departure from expectations are 0·10 (*), 0·05 (**), and 0·01 (***).

whereas nine of the ten segregating loci on chromosomes 2, 3, and 6 were deficient for the allele contributed by CM37.

A number of observations indicate that the mechanisms underlying the observed segregation distortion are pre-zygotic in nature: (a) All ears appeared full without any visible evidence of ovule abortion; (b) Seed germination was nearly perfect (>99 per cent); (c) Electrophoretic analyses were conducted on 5-day-old seedlings, prior to any opportunity for post-zygotic selection to occur. This evidence suggests that the causal processes were manifested in gametes produced by the F_1 parent, and may be broadly construed as suggesting some type of gamete competition. Whatever the physiological basis of the phenomenon, it is clear from the data of table 2 that gametic selection has occurred, and that it has a complex multilocus and multi-chromosomal genetic origin. It is additionally evident that a minimum of five genetic factors were involved, one on each of the chromosomes 1, 2, 3, 6, and 8. Because only 40 per cent of the genome was covered by the segregating markers (Edwards *et al.*, 1986), it seems probable that additional factors were present that went undetected. While the data of table 2 do not allow mapping of the detected factors, it is possible to speculate about their general chromosomal location in some cases. If one makes the simplifying assumption that all distortions on a chromosome are due to the action of a single factor, the magnitude of distortion for linked marker loci will be a function of the recombination distance between the factor and the marker. Based on the relative magnitudes of segregation distortion for multiple markers on chromosome 3, for example, one might hypothesise that one gametic factor is located near *Tpi*-4 or *Pgd*-2 (which are 10 map units apart).

Because the sexual origin of the gametes forming the F_2 could not be discerned, and because there was evidence suggesting complex gamete competition arising from factors contributed by both parents, backcross progenies were examined with the aim of partitioning the observed F_2 distortions into male and female components. Surprisingly, all loci in both families (using the F_1 as male and as female) segregated according to expectations (Table 2). We have little explanation for this apparent loss of the distortion phenomena. The sample sizes employed were sufficient to detect significant departures from expectations for many loci if skewed segregation occurred with the same magnitude observed in the F_2. Because all progenies contained the same cytoplasm, differential nuclear-cytoplasmic interactions can be ruled out. It should be noted that the F_2 and BC families were produced in different environments and seasons, with the former generated in a winter nursery in Florida and the latter generated in North Carolina in the summer. The presence of significant gamete competition in one environment and not in another suggests the possibility that environmental selection, rather than environmentally independent physiological or genetic selection, was a primary determinant of the observed segregation distortions in the F_2, and additionally suggests that its selective influence varied markedly in magnitude between the two environments. Such an interpretation would have some precedent, in that differential gamete transmission with varying environmental features has been previously noted (Herrero and Johnson, 1980; Zamir *et al.*, 1982).

One might legitimately question whether or not segregation distortion on the scale reported here is more frequent than is commonly believed. Multilocus distorted segregation frequently arises in interspecific crosses (Stephens, 1949; Vallejos and Tanksley, 1983), but has been rarely reported within a species. It seems probable that additional infraspecific examples will become evident as others examine numerous non-deleterious and ostensibly neutral markers in large progenies. We have examined a similarly sized F2 of the inbreds Tx303 and C0159, and all 18 markers fit Mendelian expectations, as did most markers in six additional F2's of average sample size 500 (data not presented). It may be that the inbreds used in the present study, T232 and CM37, which are rather divergent in pedigree, are polymorphic at a larger than average number of genetic loci influencing gamete biology.

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