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Recombination rates of soybean varieties from different periods of introduction and release

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Abstract. Theory predicts that selection for adaptability during the short term also favors selection for a reduced recombination rate in the population. The objective of this study was to test whether the cyclic short-term selection which has taken place in soybean breeding programs in the USA since the introduction of the crop has measurably reduced recombination frequencies. Thirteen soybean varieties separated into four different release periods (prior to 1940, 1940–1954, 1955–1969, after 1970) were evaluated for their recombination frequencies within three locus pairs. Recombination frequencies among the individual varieties ranged from 7.6 to 24.1% at the $p_1 r$ locus pair, from 20.9 to 30.1% at the lnp_2 locus pair, and from 28.7 to 41.6% at the $dt_1 l_1$ locus pair. Recombination frequencies were significantly different among varieties within a release period for the $p_1 r$ and lnp_2 locus pairs, but recombination frequencies did not differ among release periods for any locus pair. Thus, apparently, plant breeders have developed soybean varieties with improved adaptation without influencing recombination rates.

Key words: *Glycine max* – Plant breeding – Selection pressure

Introduction

The emphasis in breeding programs for domesticated crops is often for short-term improvement to produce genotypes giving an immediate agricultural benefit. In soybean, as well as other self-pollinated crops, this has been achieved primarily through the selection of the superior progeny resulting from biparental crosses. In most instances these can be categorized as elite crosses involving the hybridization of two superior pureline individuals followed by the search for transgressive segregates. Plant breeders face a problem in this situation. On the one hand, they desire to break linkages to allow for the substitution of inferior alleles by more favorable alleles. On the other hand, they desire to maintain all favorable gene combinations. This can be seen most clearly in a backcrossing program where one specific allele is added to an overall favorable genotype, but the same principle on a grander scale applies to two-parent crosses.

According to Maynard Smith (1978) when a population reaches genetic equilibrium in a uniform environment, selection will be towards a reduction in recombination. He concludes that there is a short-term disadvantage to recombination, since it is disadvantageous to break up adapted genotypes by recombination. Theoretical studies of linked loci in populations have led to the conclusion that there is selection pressure in favor of reducing recombination rates (Kimura 1956; Nei 1967; Lewontin 1971). As recombination is reduced, linkages become tighter. Franklin and Lewontin (1970) developed a theory of selection based on chromosome segments instead of on individual gene frequencies. Selection operating on multilocus units (Clegg et al. 1972) has been documented experimentally.

Piper and Fehr (1987) showed inferior short-term progress in soybean with increasing recombination when comparing standard breeding procedures with procedures increasing recombination through increased generations of intermating. Melchinger (1984) showed decreasing population means with increased recombination in corn. He summarized 11 other experiments which indicated a reduction in corn grain-yield with additional recombination in highly selected genotypes but no reduction, or even a slight increase, in yield with increased recombination of unselected lines.

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These reports indicate that the structure of crop breeding programs may be favoring the selection of combinations of effective chromosome segments and reduced recombination. The present study analyzed recombination frequencies of USA soybean [*Glycine max* (L.) Merr.] varieties from different breeding eras in an attempt to find evidence supporting the hypothesis that selection in a breeding program for agricultural productivity also selects for individuals with reduced recombination.

Materials and methods

Thirteen soybean varieties were selected to represent different periods of introduction or release in the USA, with the additional constraints that they were from maturity group IV and had different alleles from those found in the tester genotypes. The years of introduction or release are listed in Table 2. 'Ebony' was introduced from Korea while 'Peking' and 'Patoka' were introduced from China. 'Macoupin' was selected as a rogue in 'Mammoth Yellow', also introduced from China. These four varieties represent genotypes available for the initial phase of soybean production in the USA; genotypes from introduction and selection without hybridization. 'Chief', 'Wabash' and 'Perry' represent the first cycle of improved varieties in the USA, resulting from selection following hybridization of initial plant introductions (Leudders 1977). In fact, Patoka is one of the two parents of Perry. 'Scott', 'Kent', and 'Cutler' were considered as second cycle improved varieties, and 'Bonus', 'Union', and 'Sparks' were considered third cycle improved varieties based on the complexity of their pedigrees and the number of generations between them and the initial plant introductions (Hymowitz et al. 1977; Allen and Bhardwaj 1987).

Recombination frequencies between three marker-locus pairs representing three linkage groups (see Table 1) were determined for the 13 varieties using F_2 segregation data. Near isolines in the 'Clark' genetic background (provided by R. L. Bernard, USDA and Univ. of Illinois) were used to donate the linked alleles in the appropriate coupling or repulsion phase: P_1r (isoline L62-1377), dt_1L_1 (L72-1727), Dt_1L_1 (L68-1562) and lnp_2 (KYlnp_2). Near-isoline KYlnp_2 was selected for the recessive coupling genotype in the F_2 population from the cross L70-4049 (p_2p_2) × L62-1579 (*lnln*).

F_1 seeds were produced during the time period 1984–1989, and F_1 plants were selfed in multiple environments during 1985–1990. Control F_1 genotypes for each locus pair, Clark isoline × Clark isoline with complementary alleles, were selfed in every environment where the variety × Clark isoline F_1s for that locus pair were selfed. Because soybean recombination frequencies are sensitive to the environment during meiosis (Pfeiffer and Vogt 1989), these control populations were used to provide a correction for variation among selfing environments. Recombination was thus expressed as a relative recombination rate: defined for each locus pair as the recombination frequency of the variety divided by the recombination frequency of the Clark control cross selfed in the same environment.

An analysis of variance of the relative recombination rate was conducted for each locus pair. Selfing environments were considered as replications. Butler (1977) recommends using replicated measurements of recombination frequency to provide more realistic tests of recombination differences than can be achieved by standard errors placed on single estimates. In the ANOVA, varieties (periods) mean square was used to test for significant variation among the variety release periods. At the lnp_2 locus pair, heterogeneity among recombination frequencies for the different varieties was confirmed by chi-square analysis of recombination estimates derived by the maximum-likelihood method (Allard 1956).

In 1986, plants in the F_2 population segregating at the lnp_2 loci were classified 6–8 weeks after planting. Population size ranged from 485 to 1840 plants with a mean of 1097 plants. Populations for the dt_1l_1 *and* p_1r linkages were screened at maturity in 1988 and 1990 from selfing environments in 1987, 1988 and 1989. All varieties were selfed in multiple environments. The total number of plants classified ranged from 692 to 2658 with a mean of 1344 for the dt_1l_1 linkage group, and 1295 to 2299 with a mean of 1839 for the p_1r linkage group.

A chi-square goodness-of-fit test was performed on the classification data for both loci (except the *r* locus, see below) in each F_2 population. Six F_2 populations segregating for the dt_1l_1 locus pair produced phenotypic distributions which differed significantly ($P<0.01$) from the expected 3:1 segregation ratio for a locus. These populations were excluded from further analysis, but all varieties produced at least one F_2 population meeting the criteria for inclusion in the analysis.

Recombination frequencies for the lnp_2 and dt_1l_1 locus pairs were calculated by the product method using tables from Immer and Henderson (1943). For the p_1r locus pair, recombination percentage was estimated by the method of Kuspira and Bhambhani (1984). In previous experiments, nonpubescent P_1-individuals were underrepresented when screening was done at maturity. To overcome this problem, populations were screened during early vegetative growth for segregation at the p_1 locus. Hilum color, controlled by the *r* locus, was then determined at maturity for those populations fitting the 3:1 segregation ratio at the p_1 locus. Because the method of Kuspira and Bhambhani requires information only on the total number of individuals and the number of double-recessive phenotypes, the phenotype at the *r* locus was determined only on p_1p_1 plants. Because it was the P_1-phenotypes which were underpresented in the previous experiment, the number of p_1p_1 phenotypes scored at maturity was usually within two or three of the number counted in the initial screening. Although calculating a chi-square value for segregation at the *r* locus was prevented, this method eliminated the affect of differential loss of P_1-genotypes during plant growth on the calculation of recombination frequency.

Results

The mean recombination frequency for the p_1r locus pair was 15.2%, and variety means ranged from 7.6 to 24.1%. The mean recombination frequency for the lnp_2 locus pair was 25.7%, and variety means ranged from 20.9% to 30.1%. The dt_1l_1 locus pair mean recombination frequency was 34.9%, and variety means ranged from 28.7 to 41.6%. The overall means are all close to the standard values (Table 1), and the variety means fall well within the ranges reported for a broad-based population (Pfeiffer and Vogt 1990).

The use of relative recombination rates reduced the relative sizes of mean squares for replications (selfing environments), and in the case of the dt_1l_1 locus pair the effect of replications was nonsignificant when relative rates were analyzed as opposed to significant ($p<0.05$)

Table 1. Phenotypes of plants used to measure two-locus recombination frequencies for the soybean varieties from different release periods

Linkage group[a]	Locus pair	Recombination frequency[a]	Phenotypes
2	p_1r	20.9%	p_1, normal pubescence P_1, glabrous r, brown hilum R, black hilum
4	lnp_2	26.4%	ln, narrow leaflets Ln, wide leaflets p_2, puberulent P_2, normal pubescence
5	dt_1l_1	39.4%	dt_1, determinate growth Dt_1, indeterminate growth l_1, tan or brown pod L_1, black pod

[a] Linkage group numbering and recombination frequency from Bernard and Weiss 1973

Table 2. Relative recombination rates for three locus pairs of 13 soybean varieties released over an 80-year period

Variety	Year released	Locus pair		
		P_1R	LnP_2	Dt_1L_1
		Relative rate[b]		
Ebony	1901[a]	0.91	0.89	1.09
Peking	1906[a]	0.93	1.24	0.98
Patoka	1926[a]	0.93	0.78	0.84
Macoupin	1934	1.14	0.97	0.89
Chief	1940	0.80	0.91	0.94
Wabash	1948	1.04	0.76	0.96
Perry	1951	1.08	1.20	1.00
Scott	1958	1.55	1.11	1.16
Kent	1961	0.50	1.23	0.95
Cutler	1968	1.24	1.04	0.99
Bonus	1971	1.15	0.94	0.98
Union	1977	0.73	1.16	0.93
Sparks	1981	0.74	1.25	0.80

[a] Year introduced into USA

[b] Recombination frequency of variety crossed with Clark tester divided by the recombination frequency of Clark × Clark tester selfed in the same environment

when actual rates were analyzed. Relative recombination rates also allowed expressing recombination frequency of all locus pairs on the same relative scale. The relative recombination rates are presented in Table 2. There were significant differences ($p<0.05$) for recombination rate among varieties within release periods for the p_1r and lnp_2 locus pairs but not for dt_1l_1 locus pair. The relative recombination rates for Scott and Perry were >1 for all three locus pairs while those of Chief and Patoka were <1 for all three locus pairs. The average relative recombination rates for the four variety introduction-release periods (Fig. 1) were not significantly different ($p<0.05$) for any locus pair.

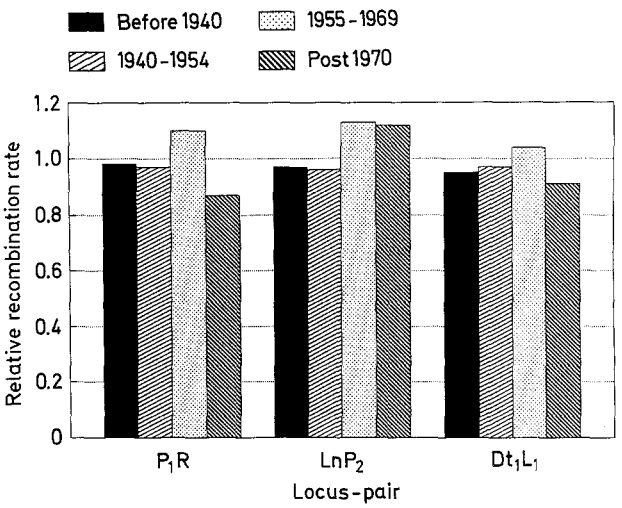

Fig. 1. Average relative recombination rates for three locus pairs of soybean varieties from different variety release periods in the USA. Relative recombination rate is the recombination frequency of a variety crossed with the Clark tester divided by the recombination frequency of Clark × Clark tester selfed in the same environment

Discussion

This study has several limitations. Soybean breeding programs have not been closed to new germplasm and, because of the additional constraints imposed on variety choice for this experiment, the varieties from subsequent periods do not have direct pedigree relationships with varieties from the previous periods. The genetic base of present-day USA soybean cultivars, however, is quite narrow (Delannay et al. 1983). Of the varieties used in the present study, Macoupin contributed significantly to the southern gene pool from the period prior to 1940 and Patoka contributed significantly to the northern gene pool of all USA varieties released between 1951 and 1960. In cultivars released from 1971 to 1981, Peking, Macopuin and Patoka are measurable, but minor, contributors to the northern and southern gene pools (Delannay et al. 1983). So, while limiting, there are genetic relationships among the varieties in this study.

Second, the Clark tester genotypes contributed 50% of the genes to the F_1 plants on which recombination was measured. Also, the chromosome segments around the evaluated locus pairs in the Clark testers were donated by different soybean genotypes during the backcrossing programs which produced the testers. Just as alleles in the variety may affect recombination frequency, so alleles from the Clark genotype or the introgressed segment

may also affect recombination frequency. If the alleles in the Clark testers produced dominant effects on recombination frequencies, no variety effects would be measurable. Any genetic relationship between tester allele effects and variety allele effects on recombination frequency is unknown. Likewise, the presence or absence in the introgressed regions of single genes controlling recombination frequency within that region's specific locus pair is unknown.

There is evidence from *Zea mays* (Tulsieram et al. 1992) and *Petunia hybrida* (Cornu et al. 1989) that single gene control of recombination can occur in plants. In many plant species, however, recombination (or, similarly, chiasma) frequency is controlled by a polygenic system and thus considered a quantitative trait (*Z. mays* L., Stadler 1926; Tulsieram et al. 1992; *Triticum aestivum* L., Rao and Murty 1972; *Secale cereale* L., Jones 1974; *Raphanus sativus* L., Dayal 1977; *Phaseolus lunatus* L., Allard 1963). Pfeiffer and Vogt (1990) employed the same Clark isolines as used in the present study to measure recombination frequencies in the AP12 soybean population, a genetically diverse population developed by the hybridization of 40 plant introductions with 40 high-yielding cultivars of maturity groups I through IV (Fehr and de Cianzio 1981). The recombination frequencies for all three locus pairs were normally distributed. Recombination frequency in the AP12 soybean population appeared to be under polygenic control. Thus, current evidence points to the assumption that genetic control of recombination frequency in soybean is quantitative.

Third, three chromosome regions are a very small sample by which to assess changes over the entire genome. The only bases for selecting these regions were the availability of testers, the single gene control of traits, and the ability to screen large plant populations in a reasonable time period. Whether or not these locus pairs are located in agronomically important chromosome regions which have been affected by selection is unknown. If they are in chromosome regions in which the selection pressure applied through plant breeding programs was inoperable, then selection pressure on recombination control in these regions would not have been present. It is also possible that the number of generations under selection was too small to effect a measurable change in recombination frequency.

Because recombination frequencies in this set of varieties were heterogeneous at all three locus pairs, and because heterogeneity in recombination frequency appears to be present in most populations in which recombination frequencies are evaluated, the potential existed for selection to alter recombination rates. In this study, reduced recombination frequencies for varieties from more recent release periods were anticipated, but there is no indication from these data that a reduction in recombination has occurred. Turner (1967) concluded that selection tends to favor an optimum, rather than a minimum, level of recombination in order to provide variability. Perhaps this occurs in soybean.

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