# EVIDENCE FOR GENETIC RESTRICTION OF RECOMBINATION IN THE LIMA BEAN

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THERE is some evidence from studies of related species of plants that low chiasma frequency is associated with outcrossing and high chiasma frequency is associated with selfing (reviews in GRANT 1958; BODMER and PARSONS 1962). From this it has been inferred that crossover rates have been adjusted by natural selection toward high values in selfing species to compensate for the restrictions imposed on the variability system by breeding habit, and vice versa for outcrossing species. The present experiment on the lima bean, a highy self-pollinated species, is reported for the bearing it has on the adjustment of mechanisms which regulate recombination rates.

Estimates of recombination between any given pair of linked loci in lima beans often differ strikingly in different hybrids and/or in different environments (ALLARD 1956; ALLARD and CLEMENT 1959). Similar fluctuations have long been known to occur in other plant species, e.g. maize (STADLER 1925). It was therefore decided to divide the experiment into two parts. In the first part, genetically uniform hybrid plants were sown, or harvested, at different times to determine the effect of various natural environments on recombination values. In the second part of the experiment, selection for high and low recombination values was practiced among selfed progeny of certain hybrids to detemine the extent of chromosomal control of recombination values.

## MATERIALS AND METHODS

Three different pairs of linked loci were studied, as shown in Table 1. Phenotypic expressions of these loci are: *R/r*—dark red *vs.* red seed-coat color; *Wl/wl*—lanceolate *vs.* ovate-lanceolate *vs.* ovate leaflet shape; *D/d*—indeterminate *vs.* determinate habit of growth; $L_1/l_1$—green *vs.* yellow plant color; *S/s*—mottled *vs.* diffuse-mottled *vs.* self-colored seed coats. These particular pairs of loci were selected for study because classification into alternative phenotypes is easy and certain. Also the three combinations *RWl/rwl*, $SL_1/sl_1$ and *WlD/wld* represent loose, moderate, and close linkage, respectively. In addition, one gene pair in each combination is partially dominant, leading to six-class segregations and hence to greater efficiency in estimating recombination values than is possible with less complete classification. Use of multiple segregating stocks would have been desirable to test whether recombination values respond independently or jointly to changes in environment, or to selection, but unfortunately suitable stocks were

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not available and their development would have been laborious and time consuming in this species.

The parental strains used (Table 1) had been maintained for many generations by growing seeds from a single selfed plant each generation. Hence, they were presumably highly homozygous. Further assurance that the $F_1$ hybrid plants studied were genetically identical was obtained by utilizing only a single plant of each parent in making each of the hybrids.

Recombination values were estimated by the method of maximum likelihood. The estimator for $p$, the recombination value, for the type of family studied is

$$\begin{aligned}\frac{d \log L}{dp} = \frac{-(e+f)(2p)}{1-p^2} + \frac{(g+h+i)(2p-1)}{1-p+p^2} + \frac{(j+k)(2-2p)}{p(2-p)} + \\ \frac{2l}{p} + \frac{m(1-2p)}{p(1-p)} + \frac{2n}{p-1} = 0\end{aligned} \tag{1}$$

where $e, f, \ldots n$ denote, respectively, observed numbers in classes $AB/AB$, $AB/aB$, $AB/Ab$, $AB/ab$, $Ab/aB$, $Ab/Ab$, $Ab/ab$, $aB/aB$, $aB/ab$, and $ab/ab$. Explicit solutions for this equation do not appear to exist but it is solved readily by iteration. The standard error of $p$ is given by $(I_p^{-1})^{1/2}$, where

$$\begin{aligned}I_p = -\frac{d^2 \log L}{dp^2} = \frac{-2(e+f)(1+p^2)}{(1-p^2)^2} + \frac{(g+h+i)(1+2p-2p^2)}{(1-p+p^2)^2} + \\ \frac{-2(j+k)(2-2p+p^2)}{[p(2-p)]^2} + \frac{-2l}{p^2} + \frac{-m(1-2p+2p^2)}{[p(1-p)]^2} + \frac{-2n}{(p-1)^2}\,.\end{aligned} \tag{2}$$

Computation of the recombination values and their standard errors was facilitated by use of a digital computer.

## THE EFFECTS OF ENVIRONMENT

*Variations between environments:* The effect of environment on recombination values was tested in each of two years by sowing three $F_1$ seeds of each of the four hybrids at a date earlier than normal (May 1), at the normal time (May 15) and later than normal (June 15), and determining recombination rates from their progenies. The results are summarized in Figure 1. Chi-square heterogeneity tests showed the data to be homogeneous within dates ($P > .05$), but highly heterogeneous between dates. Thus genetic differences among individual $F_1$ plants in combination with microenvironmental differences within planting dates had little effect, but differences amongst dates had large effects on the amount of recombination. For example, for *RWl/rwl* the maximum range of recombination values for individual families within dates was 5.70 crossover units whereas the between dates range was from 27.0 to 44.1 crossover units (Figure 1A). Attempts to associate these fluctuations with specific environmental conditions prevailing at the time of meiosis were not successful. Inspection of the results in Figure 1

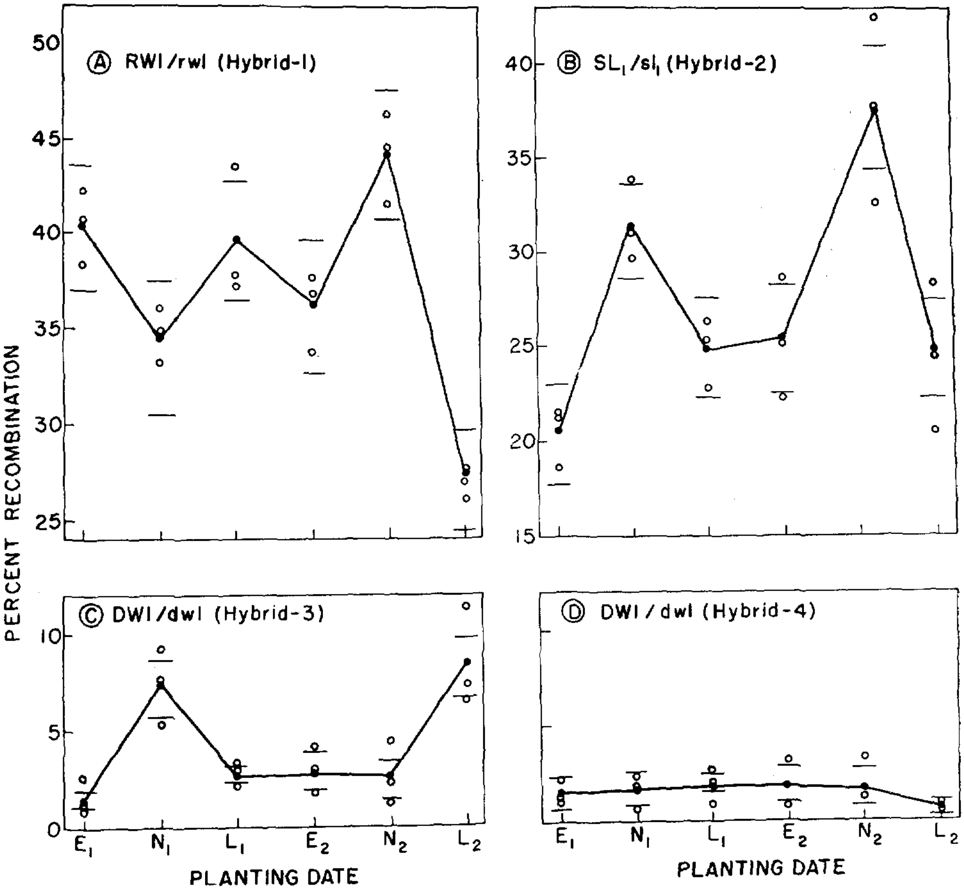

FIGURE 1.—Recombination values for genetically uniform $F_1$ plants seeded at different times. $E_1$, $N_1$, . . ., $L_2$ denote early, normal and late planting times in the first and second years. Open circles represent recombination values for single plants and solid circles represent joint estimates for each planting time. Horizonal lines give 95 percent confidence limits for the joint estimates.

also reveals no consistent pattern in the fluctuations of the four different hybrids at different planting dates, suggesting that each hybrid responds uniquely to each environment.

TABLE 1

*Loci studied and their linkage relations*

| Hybrid | Parents | Genotype | Recombination value* | Linkage group |
|---|---|---|---|---|
| 1 | L64 × L124 | *RWl/rwl* | 38.0 | I |
| 2 | L121 × L124 | $SL_1/sl_1$ | 26.9 | II |
| 3 | L63 × L48 | *WlD/wld* | 2.1 | I |
| 4 | L64 × L48 | *WlD/wld* | 2.1 | I |

* ALLARD and CLEMENT 1959.

Comparison of recombination rates for hybrids 3 and 4 (Figure 1C, D) shows that the recombination rates were higher and more variable in the former hybrid. Thus background genotype has an effect on recombination between *Wl* and *D*. However these two hybrids differed in time of floral initiation, and consequently they were not necessarily of equivalent physiological age at any given time, even though they were planted at the same time. The observed difference in recombination values may therefore reflect no basic difference in control of the chromosomal mechanism of crossing-over but merely a possibly simply inherited difference in earliness.

*Variations within individuals:* The extent to which recombination rates vary during different periods for individual plants was tested by harvesting all seeds which reached maturity during specified intervals and determining recombination values for each seed lot separately. The harvest intervals were August 25–September 12 (Period 1), September 13–October 15 (Period 2), and October 16–November 20 (Period 3). Two $F_1$ plants each were studied for hybrids 1, 2 and 3 with the results shown in Figure 2. These data were homogeneous within ($P > .05$) but highly heterogeneous amongst periods for each hybrid. Thus, in

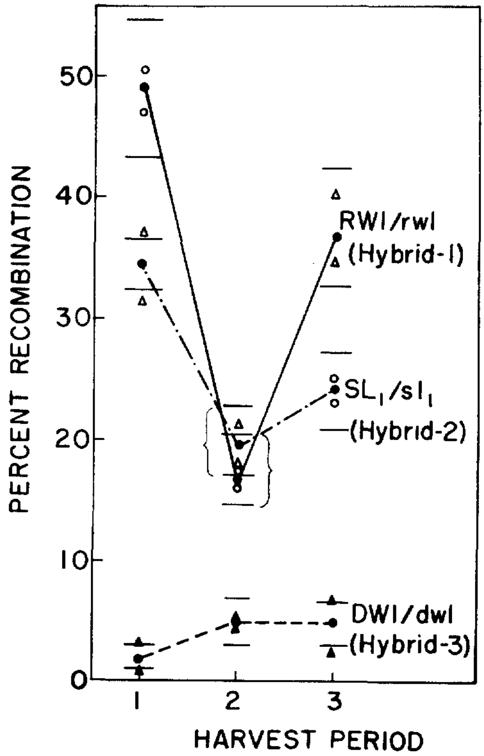

FIGURE 2.—Recombination values for samples harvested at three different times from the same plant (see text). Open circles (triangles for $SL_1/sl_1$) represent estimates for single plants in each period. Solid circles represent joint estimates for each period and horizontal lines give 95 percent confidence limits for joint estimates.

these materials, the amount of recombination varies widely from period-to-period for single individuals, providing further evidence that recombination is very sensitive to fluctuations in environment.

For *RWl/rwl* and $SL_1/sl_1$ recombination was much lower for harvest Period 2 than for Periods 1 and 3. Since meiosis occurred during the period of highest summer temperatures for seeds harvested in Period 2, this observation suggests that high temperatures reduce recombination between these two loci. However, considering the generally sporadic nature of the fluctuations in recombination rates observed in this experiment, such inferences must be regarded with caution.

## RESPONSE TO SELECTION

In the high-low selection experiment, five $F_2$ plants each of hybrids 1, 2 and 3 were chosen by random methods and the recombination value for each plant determined from the segregation ratios of its $F_3$ progeny. Three random $F_3$ plants were then taken from the $F_3$ progeny whose $F_2$ parent had the highest recombination value. Similarly three random plants were chosen from the $F_3$ progeny whose $F_2$ parent had the lowest recombination value. This was repeated in $F_4$, $F_5$ and $F_6$ generations with the results shown in Figure 3.

Inspection of Figure 3 shows that the selection lines were subject to year-to-year fluctuations of the same order of magnitude as the $F_1$ hybrids discussed earlier. However, these fluctuations do not camouflage a sharp response to selection in the high direction and little or no response to downward selection. Final mean differences between high and low selection lines in the $F_5$ generation, as measured by segregation ratios in $F_6$ progenies, were *RWl/rwl*–10.0, $SL_1/sl_1$–20.0 and *DWl/dwl*–7.5 crossover units. Nearly all of these differences resulted from response to upward selection. These consistent differences observed between the high and low selection lines in several environments indicate basic alteration of the chromosomal mechanism of recombination and not mere genotypic-environmental interaction.

## DISCUSSION

Recombination rates were found to be highly variable when stocks of high genetic uniformity were grown in different natural environments. Similarly, samples of seed harvested from the same plant at different times differed significantly in recombination values. These environmental influences often led to differences in recombination values of the order of 10 crossover units and differences greater than 20 crossover units were observed occasionally. Thus considerable caution should be exercised in comparing crossover rates of different genotypes or of different species. Numerous individuals grown in different environments and sampled at various times must be studied before it can be said that different genotypes or species have different recombination rates.

Despite the large effects of environment on crossover rates it was shown by a high-low selection experiment that chromosomal mechanisms governing recombination can be altered by selection. Even though population size was small, steady

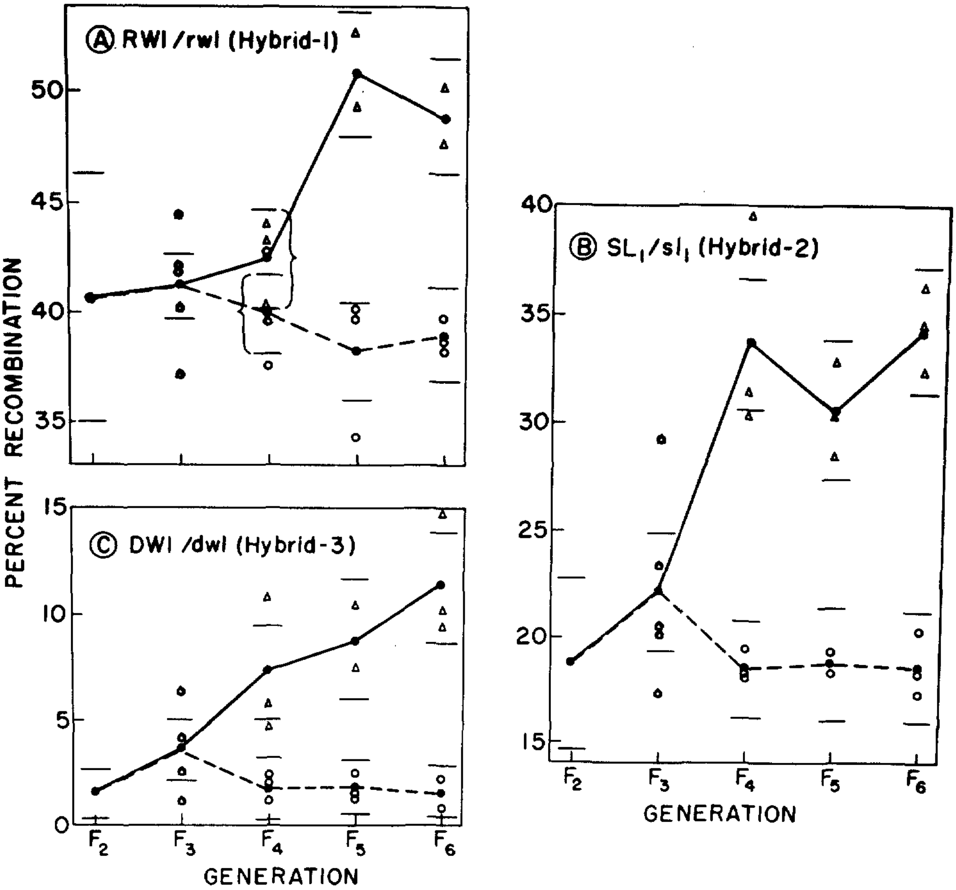

FIGURE 3.—Recombination values for lines selected for low and high recombination rates. Open circles and triangles represent recombination values for individual plants in the low and high selection lines, respectively. Closed circles represent joint estimates for each generation and horizontal lines give 95 percent confidence intervals for the joint estimates.

and rapid response was obtained to selection for increased recombination rates in each of three hybrids. The lima bean is difficult cytologically and it was therefore impractical to determine directly whether any of the observed changes had a cytological basis. However, since the responses were almost entirely in the direction of increased recombination, and cytological aberrations tend to decrease recombination, it seems likely that the changes observed resulted from altering the frequencies of genes influencing recombination rates rather than from selecting cytological aberrants. Assuming that the changes were due to genes affecting crossover rates, the steadiness of the responses suggests that more than one gene was involved in each case, and the rapidity of the responses indicates that certain of the genes involved may have had fairly large effects.

Perhaps the most interesting features of the results were first, the striking asymmetry of the responses to selection, and second, the direction of the responses.

The ineffectiveness of selection in the downward direction suggests that the genetic system in this highly self-pollinated species has been adjusted by previous selection to reduce crossing-over to a minimum. Apparently, therefore, the inbreeding habit of the lima bean does not in itself adequately suppress variability and further restriction of recombination through adjustment of the chromosomal mechanism of crossing-over is advantageous. The materials studied were cultivated types, and since great premium is placed on uniformity under cultivation, it is perhaps not surprising that these factors in the recombination system are here found in reinforcement rather than in opposition. These results thus provide further evidence that adjustment of recombination is associated with fitness in the broadest sense, but they also indicate that the adjustment reached depends on the circumstances encountered in individual cases.

## SUMMARY

Recombination values were found to be highly variable for seeds harvested from individual lima plants at different times. Similarly genetically uniform stocks differed in amounts of recombination when planted at different times in two years. These results indicate that caution must be exercised in comparing crossover rates for different genotypes and for different species.

A selection experiment for high and low recombination rates led to differences between high and low lines of 7.5, 10.0 and 20.0 crossover units, respectively, for three pairs of loci. Nearly all of the response was in the high direction, suggesting that the chromosomal mechanism is adjusted to restrict crossing-over in the lima bean, even though the mating system is one of predominant self-fertilization, which also presumably restricts recombination.

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