PART IV

BREEDING
INBREEDING COMPARED WITH RECURRENT SELECTION IN CORN IMPROVEMENT

G. F. SPRAGUE
Cereal Crops Branch, Agricultural Research Service, United States Department of Agriculture

Current developments are, to a considerable extent, a result of past history. This is particularly true in the present-day utilization of hybrid corn. The universal acceptance of the inbreeding and hybridization approach was in large part a result of the apparent inadequacies of previous methods of improving corn.

Mass selection, as a method of corn improvement, probably dates back to the beginning of domestication of corn. It must be assumed that this method was fairly effective in improving the primitive corns and in developing the wide diversity of plant and ear types, maturities, etc., which characterize the corn varieties of the world today. However, by 1910 there had developed a strong feeling among most plant breeders that mass selection was no longer effective in modifying yield. If one attempted to express this opinion in present-day genetic terminology it would be because the additive genetic variance for yield had been exhausted.

The ear-to-row breeding method was also employed at a number of experiment stations. Briefly summarized, the results with this method were quite satisfactory for such attributes as oil and protein percentage, plant and ear height and leaf area. Each of these attributes is relatively little influenced by environment. In other words phenotypic classification provides a good estimate of the genotype. This method was relatively ineffective in modifying yield. Here again the assumption appears to have been that a lack of genetic variability was at fault. With an increased knowledge of genetics at our disposal it is easy to account for the lack of effectiveness of the two breeding methods mentioned without any assumption as to a lack of genetic variability.

Since these two methods were ineffective in increasing yield, it is not surprising that, after the publications of Shull and East had appeared, a considerable interest developed in this new method of inbreeding and hybridization. After Jones developed the double cross, practical objections to use of the method appeared to have been overcome and extensive utilization was undertaken by a large number of experiment stations.

The first commercial hybrid was released in Connecticut in 1921, but the adoption of hybrid corn by farmers was very slow until better hybrids were available. Then expansion was very rapid. In Iowa, for example, less than 1% of the corn acreage was planted to hybrids in 1933. By 1943, 100% of the corn acreage was planted to hybrids.

References p. 659-660.
It has been conservatively estimated that substitution of hybrids for open-pollinated varieties was accompanied by a yield increase of at least 30%. Data from Iowa indicate that subsequent hybrid substitutions have been responsible for an additional yield increase of 20 to 30%. Possibly it should be emphasized that the inbreeding involved in the development of lines does not insure superior hybrid performance. If a random set of lines isolated from a single variety were crossed in all possible combinations and evaluated in yield trials, the mean performance of the crosses as a group would be expected not to differ significantly from the performance of the parent variety. Superior hybrid performance can be expected from only a small percentage of the possible combinations. The commercial hybrids of the Corn Belt probably involve less than 50 lines of the very large number produced and evaluated in yield trials.

It has been the common experience that significant yield increases have been much more difficult to achieve in recent years. We often hear the opinion expressed that genetic variability has been exhausted and that further yield increases will be difficult if not impossible to obtain. This opinion has caused some speculation as to type of gene action involved in heterosis and a re-evaluation of the limitations of the inbreeding and hybridization breeding approach.

Many investigators share the feeling that each of the breeding methods that has been proposed and used has some inherent limitation as to the ultimate progress that may be achieved. This limitation may or may not be related to the amount of genetic variability. In the case of mass selection, lack of progress can be related to the inadequacies of phenotypic classification and the lack of parentage control. The ear-to-row breeding method permitted a greater degree of genotypic evaluation, combining both phenotypic evaluation and a progeny test, but permitted only limited parentage control. Inadequate field-plot techniques prevented precise genotypic evaluation. Presumably this is the reason for the effectiveness of the method for attributes which are relatively uninfluenced by environment, and for the ineffectiveness for attributes which exhibit a large environmental variance.

Casual consideration would suggest that the inbreeding and hybridization approach provides adequate provision for each of these limitations. Lines are produced by self-fertilization, thus providing complete parentage control. Test cross seed can be produced in any desired quantity and therefore, through the use of randomization and replication, mean performance may be estimated with any desired degree of precision for any single yield trial. The magnitude of genotype-environmental interactions, however, may limit estimated or actual progress when tests are averaged over a production area. However, the possibility of an unlimited seed supply permits the measurement and utilization of a degree of genetic variability which would have been unattainable with earlier methods.

The first application of the inbreeding and hybridization method resulted in the production of hybrids which were markedly superior to open-pollinated varieties. Successive repetitions of the process, using either open-pollinated varieties or hybrids as source material, produced yield increases of diminishing magnitude. In theory this could result from an exhaustion of genetic variability or to changing standards for acceptability. The latter point seems to have received inadequate consideration.

To be acceptable, the first hybrids produced needed only to outyield open-pollinated varieties by a significant amount. Subsequent hybrids had to maintain or exceed this superior yield level and in addition had to possess an increased resistance to
lodging, dropped ears, the ravages of insects and diseases and desirable kernel-sizing properties, etc. If each of these attributes were conditioned by homozygosity at a single locus, then the size of population required to provide an even chance for the isolation of the desired multiple combination increases as a power of 4, the exponent being the number of attributes required for acceptability. None of the attributes mentioned above are conditioned by the homozygous condition at a single locus. Genetic information available indicates that all are quantitative traits and each is probably conditioned by many genes. Thus the real limitation may not be genetic variability as such, but rather the difficulty in handling populations of a size sufficient to ensure some degree of success.

The magnitude of this difficulty can be illustrated as follows: In an F₅ population, segregating for 10 factors pairs, a sample in excess of one million plants would need to be grown to provide an even chance for the occurrence of the multiple homozygous dominant. Even if such a type occurred, its detection would pose almost insurmountable difficulties. If the hypothetical population were segregating for 20 factor pairs, then the land area required to provide an even chance for the occurrence of the desired type would exceed 90,000,000 acres. If 30 factor pairs are assumed, then the land requirement would exceed the entire land mass of the world. It is obvious that desired types of this degree of genetic complexity can be attained only as chance occurrences. Fortunately the magnitude of the problem is minimized somewhat by the fact that each of the inbred lines used in a hybrid does not need to possess the full complement of desired alleles if some degree of dominance is involved. However, it is obvious that, for all practical purposes, the method of inbreeding and hybridization has a potential ceiling which is not imposed by an absence of genetic variability.

One of the first attempts to minimize some of the difficulties inherent in this breeding procedure was the suggestion of “early testing”¹⁰. The various recurrent selection techniques were a logical extension of the early testing procedure. These methods have the theoretical advantage in combining adequate detection and evaluation procedures with a minimum and controllable rate of approach to homozygosis.

Studies have been reported from Iowa¹¹,¹² dealing with recurrent selection on the basis of phenotype. The particular attribute chosen was oil percentage of the mature grain. One set of data reported is presented in Table I.

The parental material used was a synthetic variety designated as Stiff Stalk. Approximately 100 shoots were self pollinated and the resulting ears analyzed individually for oil percentage of the grain. The 10 ears having the highest oil percentage were used as parental material for both the selfing and recurrent selection series. In the recurrent series the 10 ears having the highest oil percentage were grown as ear row progenies and all possible combinations among them were made by hand. In the first cycle, equal quantities of each combination were bulked. This was followed by selfing within this bulk increase population. Approximately 100 self-pollinated ears were analyzed individually and the highest 10% intercrossed as outlined above.

The selfing series was derived from the same 10 ears used in the recurrent series. Approximately 10 plants were self-pollinated per progeny row. Half of these were saved at harvest and analyzed individually for oil percentage. The 2 ears from each family having the highest oil percentage were used to continue the line for further inbreeding and selection. In the second and subsequent generations the sibling progeny having the lowest mean oil percentage was discarded.

References p. 659-660.
The data in Table I indicate that seasonal effects were of some importance but were not the major cause of differences between the two breeding systems. Selection within the inbred lines was effective in increasing the mean oil percentage from 4.97 to 5.62, a difference of 0.65%. In the recurrent series the corresponding increase was from 4.97 to 7.0 or 2.03% age points. If one wishes to make a more conservative estimate the increase was from 4.2 to 7.0 or 2.83% age points. Any comparison of effectiveness of breeding systems must be based on relative costs and on gain per generation or year. These results indicate that recurrent selection was from 3 to 5 times more effective than straight inbreeding.

Jenkins et al.² reported a somewhat similar effectiveness in the transfer of resistance to leaf blight caused by Helminthosporium turcicum. These results do not permit a direct comparison between recurrent selection and inbreeding. However, they do indicate that the recurrent selection procedure was effective in concentrating genes responsible for resistance to leaf blight.

Only two studies are cited that indicate the effectiveness of recurrent selection in modifying yield. The first of these was reported by Robinson, Comstock and Harvey. This study has a bearing on several important problems. However, the one of major interest here involves the amount and nature of genetic variability in open-pollinated varieties of corn. The pertinent data are presented in Table II.

### Table I

Mean Oil Percentages for the Recurrent Series and for the Ten Selected Sibling Progenies Comprising the Selfing Series

<table>
<thead>
<tr>
<th>Series and strain designation</th>
<th>Mean oil percentage for the generation of selfing indicated</th>
<th>Total gain</th>
<th>Gain per year</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S₁</td>
<td>S₂</td>
<td>S₃</td>
</tr>
<tr>
<td>Selfing Series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5710–86</td>
<td>5.3</td>
<td>4.7</td>
<td>4.5</td>
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<td>–91</td>
<td>5.2</td>
<td>5.1</td>
<td>4.7</td>
</tr>
<tr>
<td>–10</td>
<td>5.0</td>
<td>4.8</td>
<td>5.7</td>
</tr>
<tr>
<td>–47</td>
<td>4.9</td>
<td>4.9</td>
<td>5.2</td>
</tr>
<tr>
<td>–38</td>
<td>4.9</td>
<td>4.1</td>
<td>4.4</td>
</tr>
<tr>
<td>–62</td>
<td>4.9</td>
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<td>4.5</td>
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<td>–70</td>
<td>4.9</td>
<td>4.6</td>
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</tr>
<tr>
<td>–63</td>
<td>4.9</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>–97</td>
<td>4.9</td>
<td>4.3</td>
<td>5.1</td>
</tr>
<tr>
<td>–51</td>
<td>4.8</td>
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<td>5.0</td>
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<tr>
<td>Mean</td>
<td>4.97</td>
<td>4.59</td>
<td>4.95</td>
</tr>
<tr>
<td>Recurrent Series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population mean</td>
<td>4.2</td>
<td>5.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Selected sample mean</td>
<td>4.97</td>
<td>7.17</td>
<td>8.2</td>
</tr>
</tbody>
</table>

### Table II

Estimates of Additive (σ₂) and Dominance (σ₄²) Genetic Variance and Their Ratio in Three Open-Pollinated Varieties of Corn

<table>
<thead>
<tr>
<th>Variety</th>
<th>σ₂²</th>
<th>σ₄²</th>
<th>σ₄²/σ₂²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvis</td>
<td>0.0040</td>
<td>-0.0003</td>
<td>**</td>
</tr>
<tr>
<td>Weekley</td>
<td>0.0033</td>
<td>0.0017</td>
<td>0.52</td>
</tr>
<tr>
<td>Indian Chief</td>
<td>0.0024</td>
<td>0.0008</td>
<td>0.33</td>
</tr>
</tbody>
</table>
The estimates of additive and dominance genetic variance are based on individual plant data, yield being measured by weight of ears in pounds. Estimates of additive genetic variance ($\sigma^2_a$) were positive in each case and roughly of the same magnitude. Estimates of dominance variance ($\sigma^2_d$) were numerically smaller. In one case (Jarvis), a negative estimate was obtained. This is presumably a sampling deviation from zero or some small positive value. Disregarding the case in which a negative estimate was obtained, the ratio of $\sigma^2_d/\sigma^2_a$ was less than 1.0, indicating the importance of additive genetic variance. Lonnquist has also presented evidence for a considerable amount of additive genetic variance in open-pollinated corn varieties. These data contradict the popular opinion mentioned earlier that the additive genetic variance in open-pollinated varieties had been exhausted by the selection previously practiced.

Evidence that the estimates of additive genetic variance ($\sigma^2_a$) have some validity is indicated by the data in Table III. In each case the actual gains are less than predicted. However, the agreement between prediction and observation can be considered very good when it is remembered that the “predicted gains” are subject to some upward bias due to genotype and environment interaction.

One set of data (unpublished) from the Iowa program may be used to illustrate the effectiveness of reciprocal recurrent selection. The parental sources used were synthetics, one designated as Stiff Stalk and the second as Corn Borer No. 1. Both are 16-line synthetics, and the parental inbreds were selected on the basis of stalk strength or resistance to feeding of first-brood borer rather than on the basis of combining ability. The Stiff Stalk synthetic had been subjected to yield test evaluation and found to be comparable to open-pollinated varieties of the same maturity. Comparable tests of the Corn Borer No. 1 synthetic have never been conducted.

Two cycles of reciprocal recurrent selection have now been completed. The summarized data are presented in Table IV. Each mean is based on tests grown at 2 locations for a 2-year period with 3 replications per test. All of the differences in yield are highly significant. These results indicate that the recurrent selection method has an acceptable degree of efficiency. The standard double crosses represent an average of 4 of the better double crosses currently grown in the same maturity zone. They therefore represent the achievement of approximately 35 years of effort by the standard inbreeding and hybridization technique. The reciprocal recurrent selection series on the other hand represents some 10 years of effort. These results are in agreement with the studies on oil percentage in suggesting that recurrent selection provides economics.

References p. 659–660.
TABLE IV

PERFORMANCE OF INTERCROSSES BETWEEN COMPOSITES DERIVED FROM SUCCESSIVE CYCLES OF A RECIPROCAL RECURRENT SELECTION SERIES

<table>
<thead>
<tr>
<th>Parentage</th>
<th>Yield (bu. per acre)</th>
<th>Stalk breaking (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0 \times C_0$</td>
<td>76.3</td>
<td>23.3</td>
</tr>
<tr>
<td>$C_1 \times C_1$</td>
<td>81.1</td>
<td>26.6</td>
</tr>
<tr>
<td>$C_2 \times C_2^*$</td>
<td>89.6</td>
<td>23.7</td>
</tr>
<tr>
<td>Double cross standards</td>
<td>84.9</td>
<td>27.0</td>
</tr>
</tbody>
</table>

* Average of test crosses comprising the selected sample.

for a more efficient utilization of genetic variability than does inbreeding combined with phenotypic selection.

These several sets of data lend considerable support to the validity of the theoretical assumptions underlying the recurrent selection procedures. From a knowledge of the procedures involved, it is apparent that the method provides for a controlled rate of homozygosity and a directed change in gene frequency rather than the essentially random change in gene frequencies involved in routine self fertilization. It is obvious that some degree of inbreeding is involved in both procedures.

The improved material arising from any one of the recurrent selection schemes may be used in various ways, the choice depending somewhat on local conditions: The bulk increase from a selected sample group may be used to establish a synthetic. Under some conditions this might be used directly as an improved variety. Possibly a more common practice would be to use the bulk increase as one parent in the production of hybrid seed. Finally the bulk increase might be used as source material for the production of new inbred lines.

It may be of some interest to consider the function and value of inbreeding in a corn breeding program before proceeding to more specific contrast between seed and asexually propagated species. Inbreeding performs several functions. It provides a diversity of genotypes among which selection may be practiced, it provides for the propagation of a particular genotype or gene frequency and it provides for repeatability since any desired hybrid combination can be remade at will.

The first characteristic, providing a diversity of genotypes, is often assumed to be of some importance. However, a wide diversity of genotypes exists in any random mating population such as an open-pollinated variety, synthetic or the advanced generation of some hybrid combination. Where the desired degree of variation is not provided within a single strain or variety, such variability can sometimes be obtained through crossing of desired types as well as through self-fertilization.

Several corn breeders have expressed the opinion that individual plants within a varietal population are superior to the best of the hybrids so far produced. If such plants could be asexually propagated they might well provide the basis for our current corn production.

The second function of inbreeding, propagation of a particular genotype or gene frequency, is of major importance in corn or any other species propagated solely by seed. In this connection it is desirable to distinguish between a minimum of inbreeding as opposed to inbreeding to near homozygosity.

It has been assumed by some that a high degree of inbreeding is a requirement for
desirable performance in hybrid combinations. This opinion is not well supported by experimental evidence. Jenkins and Sprague and Miller have presented data indicating that the performance of S lines in hybrid combinations is essentially the same as that of the near homozygous derivatives of these same lines. Recent reports by Osler, Wellhausen and Palacios indicate that some improvement in test cross performance has been achieved by the selection practiced during the period from S1 to S2. The gains reported were greatest in lines derived from non-adapted varieties.

The performance of double crosses has an important bearing on the behavior of crosses between heterozygotes. The single crosses used as parents are of uniform phenotype but highly heterozygous. The double cross hybrid seed produced, therefore represents zygotes produced through the combination of 2 variable gametic arrays. These variable gametic arrays differ in no important respect, except possibly gene frequency, from those produced by two heterozygous plants chosen at random from an open-pollinated variety. Thus if the right genotypes were available crosses equivalent to double crosses could be obtained by crossing two parents having no previous history of controlled inbreeding.

The third service provided by inbreeding, repeatability in crosses, is closely related to the second function, propagation of a particular genotype or gene frequency. The first hybrids released by the Rockefeller Agricultural Program in both Mexico and Colombia were based on the use of S1 lines. The use of S1 parental stocks poses certain problems in maintenance. Since each culture is highly heterozygous, the material must be retested after each propagation to ensure that no major change in gene frequency has taken place. The use of long-time inbreds, presumably genetically stable, in the United States is a convenient device to avoid the necessity for re-evaluation with each propagation rather than a requirement for acceptable hybrid performance. Thus if inbred lines are to be used, it may be more economical to inbreed to stability.

It should be apparent that the various recurrent selection schemes provide for the three inbreeding functions with a much lower level of inbreeding than required by standard inbreeding and hybridization procedures. As implied previously the importance of the 3 inbreeding functions may be quite different for asexually propagated species. These differences may be considered briefly.

Many asexually propagated species are characterized by a considerable variation in chromosome number, both aneuploidy and polyploidy, as well as genic diversity. It would appear that inbreeding is not necessary to provide genetic diversity as a basis for selection. Secondly propagation of parental stocks poses no important problem. The "repeatability" requirement is also minimized since a particular hybrid combination needs to be made only once. Thus it would appear that the functions provided by inbreeding in seed plants are reduced to a minor role in the breeding of asexually propagated species.

REFERENCES

breeding


DISCUSSIONS

A. J. Mangelsdorf (Hawaii): In your discussion with Dr. Sprague, were you able to form an impression as to what course the corn breeders might follow today if they were to start from the beginning?

H. M. Tydals (U.S.A.): Dr. Sprague comments in his paper that history makes a great difference in how things develop. In other words he implies that since the first hybrid corns showed much promise this method of breeding became very popular. There weren’t many other methods of breeding used in corn for many years. With regard to the future, while I cannot directly for Dr. Sprague, I believe it is safe to say that he feels more attention can be given to recurrent selection procedures than in the past.

G. W. Burton (U.S.A.): In reporting today on Dr. Sprague’s paper you made a statement to this effect: “Dr. Sprague has thought that had they had as precise a method for measuring yields a good many years ago as they have now they might have had different results.” He inferred that that might have been one of the reasons they did not make any better progress than they did with the ear-to-row method of testing. Is that right?

Dr. Tydals: In his paper Dr. Sprague indicated reasons for failure of mass selection or ear-to-row method was lack of precise testing of the genotype and lack of parentage control.

Dr. Mangelsdorf: I recall that the ear-to-row procedure was based upon the random pollen of the field; that is, there was no control of male parentage.

I. E. Stokes (U.S.A.): Dr. Tydals would you comment further on Dr. Sprague’s discussion of additive genetic variance.

Dr. Tydals: For simplicity let us assume a single factor affecting yield. If the effect is additive the heterozygote will be intermediate between homozygous dominant and homozygous recessive. If there is dominant gene action the heterozygote would yield higher than intermediate between the homozygous dominant and recessive. If there is over-dominance the heterozygote would yield higher than the homozygous dominant.

P. H. Dunckelman (U.S.A.): Is it true that of many inbred lines only 40 were important in the production of hybrid corn?

Dr. Tydals: The actual statement in Dr. Sprague’s paper is “less than 50”. The reason primarily is lack of good combining ability.

Dr. Mangelsdorf: Referring again to the discussion of dominance, it would seem likely that we often have favorable dominant genes interspersed with unfavorable recessive genes on the chromosomes. If so, then in crossing behavior we would expect an overdominance type of reaction as a result of linkage in the population phase. However, in this event the superiority of the heterozygote would result from pseudo-overdominance rather than from the heterozygosity of a single locus.

L. S. Wortman (Hawaii): We know that some inbreeding is necessary in corn in order to gain control of inbred lines or to get lines that we can be sure will give us good results in crossing. Do you have any ideas, Dr. Tydals, whether or not Dr. Sprague feels that we could have gotten yields as high as we have today if we had not inbred so much? In other words, suppose we had not tried to develop uniform inbred lines but had settled for only sufficient inbreeding to give us necessary genetic control and high yields of hybrids.
Dr. Tysdal: Dr. Sprague makes the statement that he has heard a good many corn breeders say that they think that there were single open-pollinated plants in a field of corn superior to any hybrids which have ever been produced by crossing inbred lines. Those good plants were the result of the natural crossing of open-pollinated heterozygous parents. With the many millions of crosses occurring this result would be expected on a theoretical basis. While Dr. Sprague indicates that selfed lines are not necessary to produce superior hybrid vigor; nevertheless in a sexually reproducing crop, it is necessary to have inbred lines in order to reproduce the desired genotypes.