TWO QUESTIONS ABOUT MASS SELECTION IN CONNECTICUT SHADE TOBACCO

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Connecticut Shade Tobacco is cigar wrapper tobacco. It is grown under cotton netting and hence it is referred to as "Shade Tobacco". Such tobacco is grown in Florida and in the Connecticut Valley. Many growers of Connecticut Shade Tobacco produce their own seed for future crops. They select plants in the field and, after the leaves have been picked, they harvest and bulk the seed from these plants. When a variety is not a pure line the method of plant selection for seed production can have a considerable influence on future crops. The objective of this paper is to comment on two aspects of mass selection relative to its effect on the variety when this is not a pure line.

Selection criteria

Selection criteria fall into two broad categories: quality and yield. Although a wrapper leaf contributes only a small amount of tobacco to the cigar it has a considerable effect on smoking quality. Furthermore, it determines the appearance of the cigar. This makes *quality* very important. In the past the *yield* was often, but not always, of secondary importance, as was observed by Hayes, East and Beinhard (1913). The enormous expense of growing Shade Tobacco has forced more emphasis on yield recently, and to ignore yield now would be unrealistic. Thus, tobacco growers and breeders have to pay attention to both quality and yield, and in so doing must consider a large number of characteristics. Some of the characteristics which can be observed in the field are: leaf size, shape and texture; internode length; angle of leaf with stalk; venation; number of leaves; sucker development; flowering time; plant vigor; and uniformity.

Disease resistance is important and often has to be considered in selection if not all plants possess resistance in the same degree. The more important diseases are tobacco mosaic, black root rot (*Thielavia*), blue mold, and weather fleck.

Quality traits are particularly difficult to describe and are usually impossible to measure. Some of them are taste, aroma, burn, texture and color. Although these individual aspects of quality are indeed very important breeding characteristics, they can scarcely be considered in mass selection at all. They come to full expression only after curing and fermentation, and they are not known to the selector who has to make his decision in the field. Furthermore, the ultimate quality of the tobacco is not only determined by the leaves themselves, but is strongly influenced by management practices on the farm and in the warehouse.

Seed production

It is relevant to our discussion to discuss the seed production process. Obviously, it is more than simply collecting a quantity of seed. In the first place, in the next generation the variety should remain unchanged and be kept 'stable'.

In the second place, it is often thought that the mass selection process might even be used to improve the variety. In such cases the borderline between mass selection for seed production and breeding for improvement becomes, then, rather vague. In practice, a 'stable' strain is a strain with little *variability*. For a commercial operator a uniform crop is of great value, because it facilitates many aspects of handling and is more profitable. It is often hoped that variability is reduced in the next generation after mass selection. Unfortunately, most Connecticut varieties are quite variable under current field management practices.

For instance, in a series of measurements undertaken on a random group of thirty plants of the variety Connecticut 49, the mean product of the length and width of the 11th leaf was 295.9 $\pm$ 41.1, or a coefficient of variation of 13.9 %. The mean length of the stalk to the 20th leaf was 60.6″ $\pm$ 5.6, or a coefficient of variation of 9.2 %.

A number of these plants were selfed and the next generation was grown. Of the next generation, the mean of the products of length and width of the 11th leaf together with the mean stalk length to the 20th leaf were determined. Again, the variability in the next generation was considerable (Table 1). This variability is phenotypic. The magnitude of the contribution of environment is unknown. That the effect of the environment can be considerable was shown by MANN and TISDALE (1956). They showed that the use of pots or separators for transplanting cigarette tobacco plants greatly reduced the variability of plant weight. Similar observations were made by us with shade tobacco by comparing plants transplanted in the regular way with plants grown and transplanted in peat pots. In one experiment the diameter at the tenth leaf level of potted plants and of regularly transplanted plants was the same, but the standard deviation of the potted plants was much lower:

3.35″ $\pm$ 0.19 for potted plants vs.
3.35″ $\pm$ 0.32 for regularly transplanted plants.

The magnitude of genotypic variability is unknown, but from experience it would also seem large. A high degree of genetic variability in Florida Shade Tobacco was noted by DEAN (1963).

Genetic variability may have several causes, even if mutations are excluded. The tobacco plant is essentially self-pollinating and produces

TABLE 1

Comparison of the means of leaf size (product of length × width of the 11th leaf and stalk length of 10 plants of the F_1 with the size (product of length × width of the 11th leaf) and stalk length of the parent plant P. All figures have been adjusted so that the column means equal 100.

Parent plant no.	Product, length × width, 11th leaf		Stalk length to 20th leaf	
	P	F_1	P	F_1
1	70	112 ± 10.6	110	112 ± 6.1
2	92	99 ± 20.1	99	107 ± 7.3
3	101	92 ± 12.8	91	98 ± 6.1
4	97	100 ± 12.8	101	99 ± 6.1
5	131	124 ± 15.2	93	104 ± 4.3
6	114	93 ± 14.3	93	112 ± 5.8
7	111	96 ± 8.1	91	86 ± 4.9
8	93	92 ± 14.1	108	95 ± 4.5
9	80	90 ± 9.5	95	94 ± 3.7
10	103	102 ± 13.5	118	92 ± 5.8
	100	100	100	100

thousands of seeds, but it outcrosses easily both naturally and artificially. In tobacco fields outcrossing can occur to a considerable extent. LITTON and STOKES (1964) found that in Burley 37 the frequency of outcrossing ranged from 1.08% to 18.95%, although in Kentucky 16 it was much less. Only when precautions are taken to prevent cross-fertilization can one be sure of complete selfing.

Before a new strain is released for commercial purposes it is usually carefully inbred for a number of generations. For instance, Round Tip was inbred for at least 10 generations prior to its release (JONES, 1921). A detailed account of the development of Burley 21 was given by HEGGESTAD *et al.* (1960), also showing a carefully controlled breeding schedule. In such cases, varieties given out to farmers are probably homogeneous and are highly homozygous.

However, because of the constant demand for new strains, it often happens that insufficient time is allowed for thorough inbreeding after the last cross or backcross, and in such cases a mixture of lines, not all homozygous, may be the result. Connecticut 49 may have been a case in point, it having been derived from a cross in 1945 (cf. SAND, 1960). It was a variable, segregating strain and it was possible to derive from it a number of very different lines that were hardly recognizable as members of the same family. Another example was the strain BR 13. This does not mean that all varieties of shade tobacco are this variable. Some existing strains, such as General Cigar's # 396 and # 911, are more uniform.

MASS SELECTION METHODS

There are several possible methods of producing seed from an established variety. The easiest manner of seed collecting would be taking of seed from a number of arbitrary plants and subsequently mixing it into a seed batch. This is rarely, if ever, done.

Another simple method would be to block off a certain portion of a field and to save the seed of all plants. A small refinement would be to eliminate obviously poor plants. This method is still practiced today. In the fall, the traveler through the Connecticut Valley can still find an occasional field with all tobacco cut, but with a dozen or so plants standing in a row and allowed to go to seed. This refers more to Havana Seed, a binder type, than to shade tobacco where this practice is perhaps only occasionally followed.

Still another method is to make a careful selection and to allow only superior plants to contribute to the seed harvest. This selection method is often used. When disease resistance is involved, the flower heads are bagged to prevent cross-pollination from other plants. Regarding the choice of field, it is believed by some that plants from outstanding fields produce better offspring than plants from poorer fields. Thus, great attention would be paid to the choice of the field from which to select seed plants.

Regarding the merits of these methods of seed production, two questions arise in the case of varieties which are not homozygous and homogeneous.

1) To what extent can these procedures be expected to keep an existing variety constant?

2) To what extent can selection pressure applied in mass selection lead to improvements in the variety?

1. Genotype of the next generation

To answer the first of the preceding questions, viz., to what degree does the next generation reproduce the parent generation, is relatively simple for an idealized situation, provided we make certain assumptions:

1) All plants produce the same number of seeds and the seeds have the same viability. Although this is never so, it may be assumed when dealing with large numbers of selected plants.
2) Use only large populations and large samples. This is nearly always the case.
3) Disregard mutations and linkage.
4) Tobacco behaves as a regular diploid. This is probably true as long as one may disregard the products of inter-specific hybridization (see Goodspeed, 1954).

Starting with a population from which a large random sample is taken for seed production, it is further assumed that the genotypic array of the sample is very similar to that of the population as a whole. In the case of self-fertilization for one locus with two alleles:

Genotypic array parent:

$$p^2AA + 2pq\,Aa + q^2aa, p + q = 1.$$

Genotypic array F_1:

$$(p^2 + \tfrac{1}{2}pq)AA + pq\,Aa + (q^2 + \tfrac{1}{2}pq)aa.$$

As the proportion of heterozygous plants decreases, the proportion of homozygous plants increases correspondingly. It is immediately obvious that the degree of change is greatest when $p = q$ and least when p or q are close to 1. Ultimately, the heterozygotes disappear altogether and fixation of characters occurs. The population becomes stable, and further selfing will not change its composition. The rate of fixation is given by the general expression:

$$(p^2 + F_n pq)AA + 2(1 - F_n)pq\,Aa + (q^2 + F_n pq)aa$$

where F_n is the coefficient of inbreeding after n generations (see FALCONER, 1964, p. 65). To determine how the changes in genotypic array influence the mean value of the population in the next generation, and assuming no over-dominance, assign the values y toAA, $\tfrac{1}{2}(y - x)$ to Aa and 0 to aa and obtain:

$$\text{Parent:}\quad py - pqx$$
$$F_1:\quad py - \tfrac{1}{2}pqx$$

The mean value of the population for this character changed in the next generation with $\tfrac{1}{2}\,pqx$. Idealized as this model is, it does indicate that without selection the next generation of plants differs from the parent populations in genotypic array and mean value for any one attribute. It further indicates that unless complete homozygosis exists the seed producer should not expect his new seed to be the same as his old if his selected plants are a random sample. However, when one deals with a highly inbred variety this effect is far less important than in cases where p is close to $\tfrac{1}{2}$.

In the case of selection pressure against one of the genotypes, a model can once again help to understand the results. Obviously, if homozygotes are favored, say AA and aa over Aa, homozygosity will be reached sooner. If, on the other hand, heterozygotes are favored, and hence homozygotes are at a disadvantage, homozygosity may never be reached. In a field, when there is no distinction among lines and where selection is within and between lines simultaneously, the distribution of genotypes can be predicted according to the formulas of HALDANE (1956). Assigning to the relative fitnesses of AA, aa and Aa the values 1–k, 1–l and 1 respectively, and to the parent generation the array $p^2\,AA + 2pq\,Aa + q^2\,aa$ we obtain after Haldane for the next generation:

$$AA:\quad (1 - k)\,(p^2 + \tfrac{1}{2}pq)/1 - k(p^2 + \tfrac{1}{2}pq) - l(q^2 + \tfrac{1}{2}pq)$$
$$Aa:\quad pq/1 - k(p^2 + \tfrac{1}{2}pq) - l(q^2 + \tfrac{1}{2}pq)$$
$$aa:\quad (1 - l)\,(q^2 + \tfrac{1}{2}pq)/1 - k(p^2 + \tfrac{1}{2}pq) - l(q^2 + \tfrac{1}{2}pq)$$

Equilibrium is reached when for Aa:

$$2pq = pq/1 - k(p^2 + \tfrac{1}{2}pq) - l(q^2 + \tfrac{1}{2}pq).$$

In other words, conditions might be such that complete homozygosity is never reached. It depends on the magnitudes of k, l and p whether equilibrium will be reached with a certain percentage of heterozygotes, or only after complete fixation. In most cases, when starting with a heterozygous population with p near $\frac{1}{2}$, the next generation will be different from the parent generation, the extent of difference being determined by the degree of disadvantage of one genotype over another. A situation as just described could be envisaged for tobacco if hybrid vigor would occur (see SMITH, 1952). The model can be extended to more alleles for each locus and to more loci.

Outcrossing has been disregarded in the previous models. Should outcrossing occur, it would be impossible to predict the genotypic composition of the next generation except in very general terms. Natural outcrossing depends on climate, insects, location and proximity to other tobacco fields, and it can assume considerable proportions. Selection efforts could be largely wasted if no measures were taken to prevent unwanted hybridization.

What has been said so far shows that there is a very good chance of not reproducing a particular seed lot, unless outcrossing is prevented and the parent population represents an already homozygous line. Also, it can be expected that the greater the degree of inbreeding, the smaller the changes in the next generation.

2. PHENOTYPE VERSUS GENOTYPE: IMPROVEMENTS

The real difficulty in a field of tobacco is to recognize the desirable genotypes from among a population of phenotypes. In the event of several fields with plants of the same variety and from the same seed lot, an additional choice of field has to be made. The problems are by no means new, and the theory of selection offers some guidance in their solution.

First, it must be recognized that only in the case of quantitative characters are these problems relevant. For characters such as disease resistance and flower color, selection usually does not offer great difficulties. As we have seen, however, most of the important attributes are of a quantitative nature. How much a variety can be improved by mass selection can be determined by means of *selection response* (see FALCONER, 1964, Ch. 11). With one locus, and only 2 alleles, the actual gain after selection can be measured by the difference between the population mean and the mean of the offspring of the selected individuals. It can be expressed as:

$$R = i\,\sigma_p\,h^2$$

R = selection response
i = intensity of selection
σ = standard deviation in phenotypic value
h^2 = heritability of attribute

The intensity of selection 'i' equals D/σ_p, where D is the selection differential, and σ_p the standard deviation in phenotypic value. This value can be computed from the formula of the normal distribution curve, and depends on the inverse of the proportion of selected individuals to total population. Hence, response R can be increased by lowering the percentage of selected individuals, or by increasing the heritability which becomes greater when the phenotypic standard deviation decreases. As KEMPTHORNE (1957) pointed out, however, this response can only be predicted if the gene effects are additive. Indications are that much of the genotypic variance is of an additive character (LEGG *et al.*, 1965) (MURTY *et al.*, 1962). Assuming that the restriction holds for shade tobacco as well, and returning to the tobacco field, the greatest response could be expected with a small number of selected plants, and the greatest possible uniformity in growth and development.

So far, we have considered only one locus. As soon as selection is made for a combination of attributes, the situation immediately becomes more complex. This is precisely the problem with mass selection. In selecting seed plants for future crops, the entire plant is judged on the merits of a number of attributes at the same time, although usually no precise information is available on whether or not these attributes are correlated. A plant is given a 'value' in comparison with the 'mean' value of the field. The words 'value' and 'mean' are now used loosely because, as a rule, actual measurements are not made. This total 'value', or selection index, is actually compound and made up of a number of weighted attributes. Furthermore, the value of each attribute consists of the sum of the genotypic value G and environmental value E. We can only learn something about the phenotypic value P and have to estimate G from P. This can be done provided variances, co-variances, and certain other parameters are known (HAZEL, 1943).

In practice, however, such a selection index cannot be used for mass selection when considering a great many individuals and a substantial number of characters. But, at least in theory, progress can be made by selection based on measurement, even if involved with many characters at the same time. Since usually we have no idea of the 'value' of a plant, selection for a number of characters at the same time remains guesswork and strictly subjective.

We may now consider the next question: Which field should be used for seed selection if a selection pressure is applied? If selections are made from a very good field, will the next generation produce as well on a poor field? Or are the better plants from a very poor stand the same plants that would be outstanding if the field were above average? By regarding the performance in different fields as the result of different characters, it is possible to obtain an idea of the so-called correlation response between these characters. FALCONER (1964) explains that when the correlation is high, the same genes control performance in different environments. Hence, it would make little difference in what environment the selections are made. If the correlation is low, it would be better to select in the environment where the population is expected to grow. For shade tobacco we do not have information on these correlated responses. Experience seems to indicate that with the low selection intensity usually applied, the field has little or no effect on the next generation. This is not really surprising. However, if the selection were severe and the intensity high, the population environment might make a difference. This possibility would have to be demonstrated and cannot be assumed.

It is clear that it is almost impossible to predict a response to mass selection when many factors remain unknown.

SUMMARY

From the general discussion of the effect of mass selection on the genotypic array of the next generation we have seen that under certain conditions mass selection can lead to homozygosity, but does not do so necessarily. Outcrossing, mutations and disadvantages of certain genotypes may prevent reaching equilibrium condition with complete homozygosity even in the simplest case of one locus with two alleles. It depends on the magnitude of these factors and the degree of heterozygosity of the variety how close to genetic uniformity we ultimately can come. The advancing of one generation has only a small effect, which becomes less as the frequency of one of the genotypes becomes less. Mass selection can reduce segregation in a variety, but only in ideal situations and only in small steps. Obviously the most effective way to promote genetic uniformity is to begin with non-segregating material obtained through careful inbreeding and within-family selection. Then, if this is available, mass selection is hardly necessary and seed collection should be done so as to prevent a return to a heterozygous condition. When only segregating populations are available, some changes can be expected if the selection intensity is low, but they will not be great and may not be noticed until after several generations.

Returning to the original question of the stability of the tobacco variety, the conclusion can be drawn that when large numbers of plants from a field are selected as seedplants the changes in the next generation as a whole will be small, regardless of the selection procedure used. When 10–30 % of all plants are allowed to produce seed we cannot expect important changes. Also, when the environmental variations are as great as in shade tobacco, the possibilities of selecting against certain genotypes for a number of characteristics simultaneously becomes virtually impossible.

Concerning whether or not mass selection can lead to improvements in the variety, it was explained that the selection intensity and the heritability of a character determine the selection response. Perhaps this response can be predicted in the case of one character, but it becomes difficult, if not impossible, to forecast the results when many characters are involved at the same time. Reference was also made to a selection index which has been effective in livestock breeding. Even if an index could be determined for shade tobacco, its use is not necessarily effective, as KEMPTHORNE (1957) pointed out. The genotype-environment interaction makes questionable the choice of certain fields over others. Without experiments, such questions cannot be answered. It is likely that mass selection with low selection pressure changes a variety very little. Again, in a field where up to 30 % of the plants are selected for seed, the pressure cannot be very great and we should not expect great changes. Only when a few plants are carefully selected for certain characters should progress become noticeable, as is the experience of single plant selection in plant breeding.

We conclude that mass selection for seed and plant breeding to improve a variety should not be confused with each other. Each has its own aims and methods, which are not interchangeable. Where uncertainty exists about seed, progeny tests usually are made to compare a seed with the parent seed. BOLSUNOV (1959) has described a number of such procedures for tobacco, though the more elaborate of them appear impractical.

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