The Indirect Service of Genetics to Breeding

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1. *Introduction*

IF we knew the action of all genes, their mutual relationship and their localization in the chromosomes, we should know what could be bred—and what could not. But this complete knowledge is not the service which genetics renders to breeding, anyhow, not yet. However, shortly after Mendelian thinking had made its way in the first decade of this century, plant breeders have expected this service. When our genetic knowledge turned out to be too incomplete to serve as a reliable basis for breeding with regard to many practically important characters, the scale has turned the other side and the conviction arose that genetics has very little, if any, value for breeding. But this is like throwing away the baby with the bath water!

Genetical knowledge is of direct value to breeding in many, though isolated, cases. But perhaps the indirect value is far greater. All modern breeding work is based on general genetical principles, in most cases without detailed knowledge of genes and chromosomes. Although plant breeders may not always be aware of this fact, their work is built on genetics.

Without minimizing in the least the importance of genetical analysis of separate characters, I have chosen as a theme for the following paper the indirect service of genetics to breeding, by which I mean the application of general principles without knowing details. Of course, it is generally known that very simple Mendelian calculations can provide us with valuable information on which to build breeding schemes, but I will make an attempt to demonstrate that more can be done in this direction, especially in difficult cases. You must excuse my choice in the first place of those cases with which I am most familiar on account of my own experience.

An introductory demonstration of the way I shall handle my subject is a discussion of mass selection versus pedigree selection. Vegetative propagation and generative testing, items 3, 4 and 5, are central principles which will be applied to different groups of plants in 6, 7 and 8. The method of repeated backcrosses in item 9 is partly a subject in itself, but will be discussed because it has a strong bearing on the foregoing.

2. *Mass selection versus pedigree selection*

With both mass selection and pedigree selection the selection itself is done individually. The difference is that with mass selection the plants are propagated as a mixture, as a mass, while with pedigree selection they are propagated separately. Of course, pedigree selection is the more complicated method and therefore plant breeders have a natural tendency to prefer mass selection. Genetics can answer the question which of the two is to be recommended.

Let us assume a population, composed of $AA+Aa+aa$. If we select for the recessive aa, mass selection and pedigree selection are equal in value, simply because all progenies of aa are the same and mixing similar groups does not change the composition.

However, if we select for the homozygous dominant AA, the situation is different. Three cases can be distinguished:

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1. *Self-fertilizers.* Since the recessive *aa* can be recognized and eliminated, the difficulty lies in distinguishing between *AA* and *Aa*. Pedigree selection yields:

$$\begin{aligned} AA &\longrightarrow AA \\ Aa &\longrightarrow AA+2\,Aa+aa \end{aligned}$$

Hence the offspring immediately reveals which is which and we reach our aim completely. Mass selection would mean a mixing of the above two groups which yields—with the same number of offspring from *AA* and *Aa*—by adding: 5 $AA+2\,Aa+aa$. We are better off than in the original population, but do not completely reach our aim.. Further calculations show that in future generations the relative increase of *AA* is fairly slow, while theoretically the complete elimination of *Aa* is never reached.

2. *Cross-fertilizers which can be evaluated before flowering.* The recessive *aa* can be eliminated in time, so that the population $AA+Aa$ remains and the pollen mixture in this population is $3A+a$. Hence we get, first with pedigree selection and by adding with mass selection:

$$\begin{array}{rcll} AA & \longrightarrow & 2\,A\,(3A+a) & =6\,AA+2\,Aa \\ Aa & \longrightarrow & (A+a)\,(3A+a) & =3\,AA+4\,Aa+aa \\ \hline AA+Aa & \longrightarrow & (3A+a)\,(3A+a) & =9\,AA+6\,Aa+aa \end{array}$$

Assuming that the original genotypes are no longer available, when their progenies are known, neither pedigree nor mass selection leads completely to the goal. But the ratio of $AA : Aa$ is much more favourable with pedigree than with mass selection, namely, 3 : 1 instead of 3 : 2. Consequently the former method is recommended.

3. *Cross-fertilizers which cannot be evaluated before flowering.* In this case the pollen mixture in the population is $A : a$ and a similar calculation as above is as follows:

$$\begin{array}{rcll} AA & \longrightarrow & 2\,A\,(A+a) & =2\,AA+2\,Aa \\ Aa & \longrightarrow & (A+a)\,(A+a) & =\;\;AA+2\,Aa+aa \\ \hline AA+Aa & \longrightarrow & (3A+a)\,(A+a) & =3\,AA+4\,Aa+aa \end{array}$$

The results are similar to those of the former case, but pedigree selection does not outvalue mass selection in the same degree, namely, 3 : 3 instead of 3 : 4.

The general conclusion is that pedigree and mass selection have the same value for recessive characteristics, while in all cases pedigree selection is better than mass selection for dominant characteristics. In practice we seldom know exactly whether the genes which govern the characters for which we select are dominant or recessive. But usually our characters are multigenic and some of the genes will be dominant, others recessive. Therefore it will always be safe to apply pedigree selection. This is in perfect harmony with the evolution of breeding methods which goes from the mass to the individual.

Calculations like the above are ridiculously simple and we usually have to deal with much more complicated cases. But if we start from populations with other compositions, the results remain principally the same. Therefore it is permissible to base our work on simple cases.

3. *Vegetative reproduction as a cultural method*

Once we have produced a selection from a population, the problem of reproduction as true to type as possible arises. However, in plants which are propagated vegetatively this problem is no problem, because *vegetative reproduction maintains the genotype of the selected plants completely.* It is unnecessary to make the selection homozygous and in almost all cases vegetatively propagated plants are heterozygous. We could indicate a clone as a number of iso-heterozygous plants.

It is more or less for the sake of completeness that this point has been discussed, but three additional remarks should be made.

Annual plants propagated vegetatively may have this in common with perennials, that a certain genotype bears flowers and fruits more than once. This is important from the breeding standpoint, as will be explained later on. By applying vegetative reproduction in annuals which normally are multiplied by seed, these annuals are made into perennials and this has certain advantages.

Doubtlessly many varieties of our garden plants, like ornamental trees and shrubs, perhaps also plants like Strawberries and the Potato, are heterosis crosses which by means of vegetative reproduction keep their valuable characteristics.

The ease of vegetative reproduction may have the disadvantage of being too easy. The breeding of new varieties of plants like the Rose, the Strawberry, the Dahlia, the Apple—just to mention a few rather arbitrarily—frequently stops with the first generation of a selfing or a cross, while another generative generation might contain far better recombinations of genes. The usual breeding of vegetatively propagated plants is too static and should be more dynamic.

4. *Vegetative reproduction in plants which are normally propagated by seed*

Because vegetative reproduction is such a great help in obtaining a progeny completely identical with the selected plant, it is self-evident that attempts are made to apply vegetative reproduction in plants which thus far are propagated by seed. Several tropical plants, like Hevea, Coffee, Tea, Cocoa and others, could be mentioned as examples. In many of our horticultural plants vegetative reproduction is possible in some way or other, but it is too expensive compared with seed growing to be applied in ordinary practice. However, as part of a breeding scheme, it may be of immense value. Without intending to be exhaustive I mention three cases in which vegetative propagation would be a help to the breeder.

Very valuable plants of which little seed is available can be increased in number by vegetative propagation, so that more seed can be grown.

Selection of individual plants can be replaced by selection of clones which, of course, is much more accurate. A prerequisite is that the clonal plants develop their typical characteristics in the same way as their mother plants do and this is not always the case.

It may be that the first seed generation of certain plants, either after selfing or crossing, yields excellent results, while the next generation breaks down. Vegetative propagation of these mother plants might enable us to grow good seed generations perpetually. A special case would be the maintenance by means of vegetative propagation of the parents of a very valuable heterosis cross.

I shall deal with the most important application of vegetative propagation as part of a breeding scheme in detail further on. It is the vegetative maintenance of certain genotypes until we have tested their generative progenies which is especially important in annual or biennial cross-fertilizers.

To conclude this section, I would like to say a few words on the possibility of vegetative propagation. The thesis could be held that each plant can be propagated vegetatively, but that as yet we do not know the most suitable method in certain cases. Having recognized the value of vegetative propagation for breeding, it then becomes feasible to work out methods for this means of reproduction. Two cases will probably have to be distinguished, the permanent and the temporary vegetative propagation. By permanent I mean that vegetatively propagated plants can be vegetatively propagated again indefinitely, which will usually be the case in perennials, including herbaceous perennials. By temporary I mean that vegetatively propagated plants cannot be propagated vegetatively again, because they start to flower, to bear fruits, and die, which usually happens in annuals and biennials. In this case the vegetative period should be increased by suppressing flower initiation, and developmental physiology teaches us how to reach this objective. Temperature and length of day are limiting factors and much can be done by regulating them. It is tempting to tell about the results which my collaborators and I have obtained with several plants. However, the service of developmental physiology to breeding is not my subject to-day, and therefore I shall come back to my theme by answering the question: What can be reached by introducing vegetative propagation as part of a breeding scheme?

Since generative testing very closely goes together with this vegetative propagation, I must say a few words on generative testing first, however.

5. *Generative testing*

The genotype of a plant can only be determined by studying its generative progeny, either obtained after selfing or after crossing with a known genotype. This very fundamental principle of genetics has also become a very fundamental principle of breeding. The only difference is that the breeder does not speak of 'genotype' but of 'breeding value'. All breeders know that the breeding value of a plant can only be determined by growing the next generation. We call this 'testing', and since I speak of seed generations we call it 'generative testing'.

This testing is somewhat similar to a chemical reaction. The question: 'What happens if we add an acid or an alkali?' is translated to: 'What happens to the progeny if we self a plant, if we cross it with a known recessive, if we let a mass of cross-fertilizers intercross?'

Selfing is a very easy and very efficient way of testing self-fertilizing plants. It immediately reveals which original genotypes have been homozygous and which heterozygous:

$$AA \longrightarrow AA, \text{ but } Aa \longrightarrow AA+2\,Aa+aa$$

In cross-fertilizers, self-fertilization might be applied, but usually leads to undesirable side results. Test crossing each plant individually with a known recessive is a good method:

$$AA\times aa \longrightarrow Aa, \text{ but } Aa\times aa \longrightarrow Aa+aa$$

However, this method would be very laborious, apart from the difficulty of obtaining the recessive wanted. The principle of a much simpler method which I originally named *mass test crossings*, and which consists of a general intercrossing of all plants in a population, is as follows:

Since the difficulty lies in distinguishing between AA and Aa, we might start from a population which is composed of $AA : Aa$. The gametic ratio in this population is $3\ A : a$. Hence the progenies of AA and Aa after complete intercrossing are:

$$AA \longrightarrow 2\,A\,(3\,A+a) \longrightarrow 6\,AA+2\,Aa$$
(no visible segregation);

$$Aa \longrightarrow (A+a)\,(3\,A+a) \longrightarrow 3\,AA+4\,Aa+aa$$
(visible segregation).

Consequently we can recognize AA and Aa from their progenies, and the same principle holds true when we start from more complicated populations.

Frandsen in Denmark and Tysdal *et al.* in U.S.A. have independently developed a method which they call 'polycross' and which for the main part is identical with mass test crossings. In so far as polycrossing is definitely meant as a testing method, while the polycrossed progenies are not used for further selection, I prefer the term 'polycross' to 'mass test crossings', but in order to indicate clearly the nature of the procedure it is advisable to speak of 'polycross test'.

The polycross test in itself is very similar to ordinary pedigree breeding, but in the latter case the polycrossed progenies of individual plants are used for further selection and not as a test only. The most efficient use of the polycross test, of course, is in the breeding of cross-fertilizers, and this will be discussed in 8.

To summarize this section it can be said that generative testing in self-fertilizers is done by growing a selfed generation of each plant individually. In cross-fertilizers it is done by polycrossing and, again, by growing the next generation of each plant individually as a test.

6. *Breeding perennials without vegetative propagation*

The testing is done either by selfing or by polycrossing, as the case is. When the testing has been completed, we might use selfed progenies which are very uniform for further work. However, if loss of vigour is to be expected and also if polycrossing has been applied, the mother plants of the very best progenies are chosen for growing the bulk of seed for practical purposes.

This method is in principle: testing the genotype and growing seed from genotypes which have proved to throw a very desirable progeny.

I have applied this method in Cyclamens. Also, one of the Aalsmeer breeders of Anthurium is applying the method, and although not working on so large a scale he got striking differences in the first generation from selfing.

7. *Breeding annual self-fertilizers*

The term 'annual' includes 'biennial'. From the breeding standpoint the typical characteristic of both groups is that they bear flowers and fruits only once. This implies that a breeding scheme as dealt with in 6 is out of order.

The breeding of annual self-fertilizers is relatively easy. It is generally known that a population after continuous inbreeding is composed of a mixture of mainly homozygotes. This is what Mendelian genetics has taught plant breeders, and they apply it by growing the selfed progenies of a population during a number of generations, say five or six, without any selection. Next, the desirable plants are selected and there is a very good chance that they are homozygous and are the starting-point for pure lines. This has to be tested, which is done by simple pedigree breeding.

In cases where we know something about the genetics of the characteristics for which we select, it is possible to start the selection earlier, even in F_2. As a practical subject for students we are breeding a very early Sugar Pea with wrinkled cotyledons by crossing a late, high-yielding, smooth-seeded Sugar Pea with very early, wrinkled seeded, parchmented varieties. As early as in the F_2 selection for earliness, wrinkled seededness and lack of any parchment starts. A F_2 of some 2,000 plants yields enough desirable plants to be used as a starting-point for inbreeding during some generations, followed by final selection for yield. However, such cases, in which genetics serves breeding directly, are relatively rare.

I wish to point out the similarity and the difference between vegetative reproduction and the effect of continuous self-fertilization. Both lead to a complete reproduction, the former, however, as a heterozygous clone, the latter as a homozygous pure line. Vegetative reproduction can be applied in any stage of breeding, while a pure line is only obtained after a number of selfed generations.

8. *Breeding annual cross-fertilizers*

There was nothing new or even worth paying much attention to in the discussion of the self-fertilizers in 7. This is different with the cross-fertilizers, which group offers great difficulties and therefore is much more interesting from the standpoint of breeding science.

If we have obtained a F_2 population composed of $AA : 2\ Aa : aa$, the gametic ratio is $A : a$, both for the egg cells and for the sperm cells. Hence the F_3, obtained without any selection, is $(A+a)\ (A+a) \longrightarrow AA+2\ Aa+aa$, which is exactly the same as the F_2. In general, without selection—and with a few minor restrictions—$F_n = F_2$. The consequence is that there is no reason to wait with selection longer than the F_2, but the obtaining of true breeding—homozygous—plants is extremely difficult. Mass selection is of very little value. Pedigree selection proceeds but slowly and may lead to loss of vigour due to inbreeding.

Modern methods have been described in literature and as such I mention complete inbreeding by selfing, followed by heterosis crosses. This is an admirable method which, however, is somewhat out of order in my general line of discussion and which I will not mention further.

Furthermore, in certain cases pair crosses, diallele crosses and the remnant seed method have been developed, but they all have certain disadvantages. Pair crosses soon lead to inbreeding-deterioration. The method of diallele crosses is certainly most elegant, but can only be applied practically on too small a scale. The remnant seed method is not much more than a slight improvement of pedigree breeding.

It seems as if much can be done by maintenance of a number of genotypes until they have been generatively tested by polycrossing. This is where vegetative reproduction—item 4—enters the scene:

1. For maintaining the genotype by dividing each clone into two parts, one of which is used for the polycross test, the other one for maintaining the genotype in some or other way;
2. For a considerable improvement of the efficiency of the polycross test, since the clonal plants can be mixed thoroughly when being planted out.

The maintenance of the original genotypes needs a somewhat more detailed discussion for three different cases according to whether we deal with plants which can be (1) vegetatively propagated permanently, (2) vegetatively propagated only temporarily, but which can be selfed, (3) vegetatively propagated only temporarily and cannot by any means be selfed.

If permanent vegetative reproduction is possible, there is no difficulty, for we can easily go back to the original genotypes after having tested them. The method is very similar to the one discussed in item 6. The advantage of vegetative propagation is that reproduction on a much larger scale becomes possible.

In the case where only temporary vegetative reproduction is possible, but self-fertilization can be applied, the course of events is as follows. After vegetative reproduction of a number of selected plants, each clone is divided into two parts. One part is used for the polycross test, while the plants of the other part are selfed. The seed from these selfings is kept until the results of the test crossings are known, leading to a final selection of original genotypes. The latter can be supposed to be homozygous with regard to the characteristics for which the selection was done, if the method has been completely effective. Hence the selfed seeds of these genotypes are iso-homozygous. Propagation of these seeds, therefore, leads to complete reproduction.

Some additional remarks should be made. There is no danger of undesirable results on account of inbreeding. If there were immediate deterioration in the seeds of the selfed clonal plants, it is of no importance, since the plants from these seeds are not used for selection. Furthermore, mixed propagation of a number of generative progenies from selfed clonal plants will immediately undo eventual deterioration.

It can be expected that many cross-fertilizers will set some seed after selfing. We know some methods of increasing the setting of self-fertilized seed, like bud pollination, treatment with growth hormones, regulation of temperature, as described by several research workers. We only need a fairly small quantity of seed for maintaining certain genotypes. Since this seed is kept for some time in store, this part of the method is similar to the remnant seed method. The principle is quite different, however, and therefore I call it 'modified remnant seed method'.

If self-fertilization is impossible by any means, we are brought to the third of the above-mentioned groups. In this case the most simple method of retaining certain genotypes seems to be pair crosses. Our hope then is that some pair crosses occur of which both partners will pass the polycross test satisfactorily. Of course, this chance is small and therefore self-fertilization is preferable, even if only possible after much effort.

Without vegetative reproduction the method is not impossible. We then must use part of the flowers of one plant for polycrossing, part for selfing or pair crossings. Such a method would be very laborious and almost impracticable. This is why research on methods of vegetative propagation of cross-fertilizers is so extremely important from the breeding standpoint. Also, if only temporary vegetative reproduction is possible, methods for obtaining selfed seed are important.

The method for breeding cross-fertilizers, as described above, is fundamentally rather different from the ordinary pedigree breeding. It necessitates a new class of plants with regard to their breeding scheme: cross-fertilizers which, in a breeding method, can be propagated vegetatively.

9. *The method of repeated back crosses*

There are two reasons why I like to discuss the method of repeated back crosses. First, because too little attention is paid to this extremely valuable method; second, because the general applicability is considerably increased by applying the above-described principles of testing.

Many of our varieties are 'almost' ideal, almost, because they frequently lack one or two important characteristics. If these bad spots could be replaced, a real improvement would have been obtained, and this is the object of the method of repeated back crosses.

In many cases resistance is not only a very desirable, but also a very much desired characteristic. It may occur, but frequently in varieties which possess a large number of undesirable characteristics in addition to the resistance. An ordinary recombination cross would require enormously large numbers of plants. By back crossing this number can be reduced considerably. If we compare a F_2 with one back cross the reduction of necessary plants is in the ratio of $4^n : 2^n$. If we compare a F_n with n–1 back crosses the reduction is in the ratio of $2^n : 2$.

As a special example I mention scab resistance in Apples. Although varietal differences with regard to degree of susceptibility occur, complete resistance is unknown in commercial varieties, but it occurs in species like *Malus floribunda* which readily cross with the cultivated Apple. If our object is the breeding of an Apple like 'Cox's Orange Pippin', but resistant to scab, the course of events would be as follows. The F_1 of the cross 'Cox's Orange Pippin' $\times$ *Malus floribunda* is back crossed with Cox. In the progeny of this back cross we select for resistance and only for resistance. The resistant plants again are back crossed with Cox, and the story is repeated until after a number of back crosses, say five, our aim is reached. Of course, there are several difficulties to be overcome of which probably the most important is the reduction of the period from flower to flower. In several ways attempts can be made to solve these difficulties, but it would be too far from my proper subject to discuss them.

If the characteristic for which we select is dominant, so that the back crossing is done with the recessive allelomorph, everything goes very smoothly. The original cross is $AA \times aa \longrightarrow Aa$, and the first back cross is $Aa \times aa \longrightarrow Aa + aa$. We can immediately differentiate the latter two types and immediately proceed to the next back cross $Aa \times aa$.

If, however, the characteristic for which we select is recessive, so that the back crossing is done with the dominant allelomorph, difficulties arise. The first back cross is $Aa \times AA \longrightarrow AA + Aa$. We want to make our second back cross with Aa, because this type contains the desired recessive gene, but we cannot recognize it from the undesirable AA. In literature it is advised to apply self-fertilization for one generation. Aa then will segregate into

$AA+2\ Aa+aa$ and the back crossing is done with *aa*. However, this means loss of a generation and loss of very valuable time. Fortunately, this is unnecessary, for a simple trick enables us to continue with the back crossing without loss of time. It will be as well to discuss this solution for both self-fertilizers and cross-fertilizers.

The problem is the distinguishing between *AA* and *Aa* and, of course, this is a problem of testing. In self-fertilizers the testing is done by selfing; in cross-fertilizers by polycrossing. This is no news. But we can simultaneously do the back crossing and the testing, if only the plants bear more than one flower. For some of the flowers of one plant are back crossed, while others are used for testing. After the testing is completed, we decide which back crosses have been of the desirable type and we go on with these, discarding the others.

The extra work in this procedure is that we make twice as many back crosses as are necessary, but the gain is the time of one generation!

Another difficulty might arise, if the characteristic for which we select does not demonstrate itself before flowering. Again, we make a number of back crosses and later on select those for further work which after flowering have turned out to be the good ones.

SUMMARY

The indirect service of genetics to breeding has been illustrated by a discussion of:

1. Mass selection versus pedigree selection, having similar effects for recessive characteristics, while the latter always is more effective for dominant characteristics.
2. The value of introducing vegetative reproduction as part of a breeding scheme.
3. The principle of generative testing, which is practised by selfing in self-fertilizers, by polycrossing in cross-fertilizers.
4. The breeding of perennials, by returning to the original genotypes after generative testing.
5. The breeding of annual self-fertilizers, by inbreeding during some generations, followed by selection and testing.
6. The breeding of annual cross-fertilizers, by introducing vegetative reproduction, using parts of each clone for a polycross test, while the other parts are used for maintaining the genotypes either as vegetative clones, as seed of selfed clones, or as seed of pair crosses, as the case may be.
7. The general applicability of the method of repeated back crosses, also in 'difficult' cases, by making test selfings or polycrosses simultaneously with new back crosses.

The conclusion can be drawn that breeding can make a wider use of genetical principles than nowadays. In practice this means considerably more technical and administrative work for the breeder. However, horticultural breeding needs methods which lead as soon and as completely as possible to the goal. At a meeting of plant breeders some years ago I compared the use of methods of different levels with travelling by bicycle, by motor car or by aeroplane. They all take us where we want to be taken . . . if we have the time available. But we are living in the century of the aeroplane and if plant breeding is to keep up to date it has to make use of aeroplane methods.

I must say a word on the fact that the practical application of the methods which I discussed should not be underestimated. It is much more complicated than simple schemes would lead us to expect. But the service which genetics gives us is the underlying principle and that is the most important.

No two plants can be bred according to exactly similar methods andls detai for each separate plant will have to be worked out. The attractiveness of al! scheming and classifying is that finally each plant has its own secrets.

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