SELF-STERILITY, SELF-FERTILITY, INBREEDING AND HETEROSIS: GENETICAL INTERPREATION, AND APPLICATION IN PASTURE PLANTBREEDING.

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It is not the object of this paper to supply specific information on the behaviour of pasture plants under various conditions of fertilization. I shall attempt to review some of the results obtained in work on various organisms, in the search for those principles which are fundamental to the phenomena forming the subject of this paper; fundamentals which, I admit, may not generally allow of a direct application in economic plant breeding, but which may lead the way to new ideas and to new methods of procedure.

There is one idea fundamental to modern crop biology: that the biological constitution of any crop should be controlled. Needless to say, this does not imply that all crops should be uniform, consisting of one pure line of one species. But whether a plant population consists of one variety of one species - as in most agricultural crops - or of an association of a number of species - as in most pastural crops - we ask that any individual Component, species, variety or strain, be biologically "controlled", i.e., its members should be reasonably uniform in a genetic sense. It appears to me that our present ideas are perhaps not the last word on crop constitution; that even in our agricultural crops; particularly in those with dense spacing, plant associations of closely related forms might be superior to the single lines constituting our present crop varieties. But this would only on the surface mean a reversion to the mixed crops of days gone by: each constituent would be biologically stable, its performance known; alone and in co-operation with others, and the composition and relative prevalence in the mixture would be controlled...

GENETIC HOMOGENEITY:

For the achievement of genetic uniformity, no method of mating is superior to inbreeding. It increases most rapidly the homogeneity of the material, producing nearly complete homozygosity in about 8 generations; it induces differentiation, and thereby facilitates the elimination of undesirable, and the selection of superior types.

Inbreeding is an ideal method, where Nature will allow its application. But in many cross-fertilizing organisms, including most pasture plants, its use is largely restricted by two phenomena not necessarily inter-connected: self-sterility, and loss of vigour.

SELF-STERILITY AND SELF-FERTILITY:

When in an hermaphroditic organism its own male and female sexual cells, though functional in combination with other individuals, are unsuccessful when mated with each other, we call this phenomenon self-sterility, or self-incompatibility. In the earlier stages of investigation it was thought that this was due to some hostility toward self-fertilization, as such; as Darwin put it, "a self-sterile plant can be fertilised by the pollen of any one out of a thousand or ten thousand individuals of the same species, but not by its own." Since then overwhelming evidence has been brought forward to show that this is not generally true. In the majority of cases investigated these is a well defined group behaviour, with intra-sterility and extra-fertility, i.e. members of the group are sterile amongst themselves and fertile with members of other groups. These groups are well defined, and, as has been shown in a number of plants belonging to different species and families, they are based on genotypical differences. In their classic experiments on Nicotiana, East & his collaborators have shown that self-sterility is due to the action of a series of fifteen allelmorphs S_1 to S_{15}, which are responsible for all phenomena of group sterility in Nicotiana. It is considered likely that the number of allelomorphs actually in existence is not much larger, perhaps about 20. The action of these sterility genes is peculiar. If one line carrying $S_1 S_2$ is crossed with another one carrying $S_1 S_3$, only the pollen carrying S_3 is functional with S_1 and S_2 ovules:

$S_1 S_2$ ♀ X $S_1 S_3$ ♂ .... $S_1 S_3$ and $S_2 S_3$

ovules: S_1, S_2 functional pollen : S_3

similarly, the reciprocal cross produces :

$S_1 S_3$ ♀ X $S_1 S_2$ ♂ .... $S_1 S_2$ and $S_2 S_3$

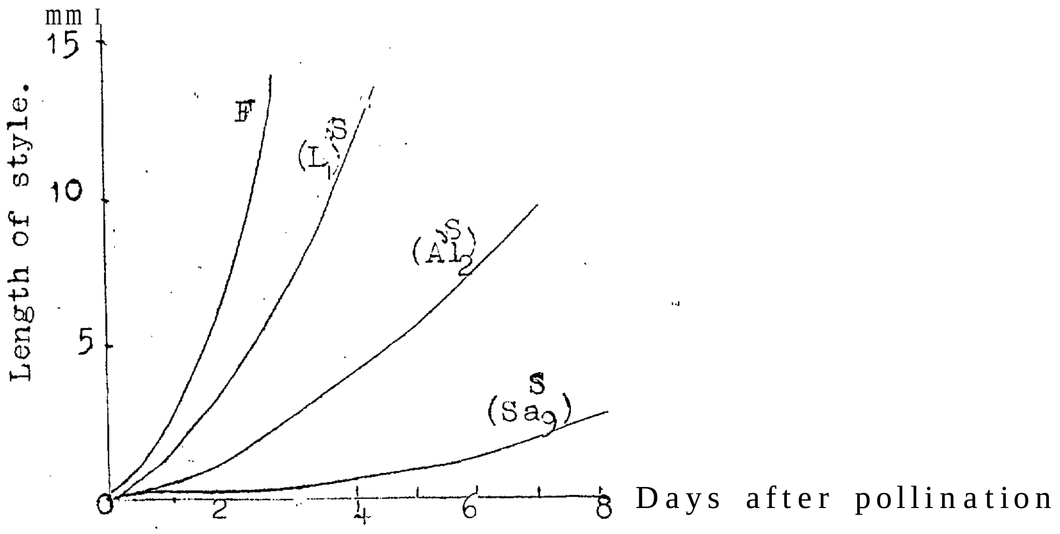
Since only pollen with sterility genes not carried by the mother plant can function, selfing is ordinarily impossible.

This theory has been tested in various ways, the most convincing probably being the establishment of equal linkage relations for various sterility genes with one gene for corolla colour which is located in the same chromosome.

The species Nicotiana Langsdorff'i, which is self-fertile, has been shown to carry a fertility factor S_f, which is allelomorphic with the sterility genes, and dominant in relation with them. $S_f S_f$ or $S_f S_1$ accordingly are self-fertile.

In addition to these genes, there have been shown to exist a number of, probably recessive, genes causing "pseudo-fertility". Brieger has shown that one such factor, P, in the presence of certain among the sterility factors, induces normal seed production on self-fertilization. Pseudo-fertility in this case is determined genetically. It can, however, also be caused phaenotgpically, viz., by the presence of such environmental conditions which are particularly favourable to fertilization. Under such circumstances ordinarily incompatible matings, may become partially fertile. In Nicotiana, fertilization in the bud, several days before flowering, and fertilization towards the end of the flowering season, have been most successful,

Phaenotypic pseudo-fertility is easily understood if the physiological basis of compatibility and incompatibility is studied. It has been shown that the difference is due to different rates of pollen tube growth. In both, compatible and incompatible matings, pollen tubes grown in the tissue of the. style, but in the former growth is faster than in the latter. In Nicotiana, the normal life of a flower is about 8 days. If the pollen tube does not reach the ovule by this time - if it is, so to speak, too late in arriving - no fertilization occurs. Brieger found the following growth rates of pollen tube growth of fertile (F) and sterile (S) pollen:



It will be seen that the F line has a more rapid growth curve, particularly in the latter part, whereas the S lines are almost straight. The differences anong the self-sterile lines are explained with the action of subsidiary genes. But it is also likely as pointed out by East, that various S genes possess different physiological values, since some seem to be more potent than others, perhaps each of the S factors having a typical pollen tube growth rate of its own. It will be further understood why environmental circumstances may exert a strong influence on compatibility: There is not a qualitative, but a quantitative difference - rate of growth - between success or failure of fertilization. Environmental factors favouring pollen tube growth, such as high temperature or high cell-sap concentration, may speed up the process sufficiently to render it successful, where less favourable conditions would lead to abortive growth. Finally, East suggests that the modifying genes which determine pseudo-fertility may act through a shortening of the style, or lengthening of the "life" of the flower, etc.

Work on other plants revealed similar mechanisms for self- and cross-sterility and fertility: group behaviour, based on the action of sterility genes; pseudo-fertility resulting from the action of modifying genes; and pseudo-fertility under the influence of the environment.

No genetic analysis of compatibility has been worked out in any of the pasture plants. The material available, however, seems to permit certain inferences from the general principles disdovered in organisms more accessible to genetic experimentation.

The existence of large individual differences in self-compatibility has been established in all cross-fertilizing pasture plants. In species with a high average proportion of facultative self-fertilization, such as Cocksfoot, as well as in species with a high average degree of self-sterility, such as Red Clover, or Perennial Rye-grass, individuals have been found with absolute self-sterility, as well as others with a high degree of self-fertility. The technical difficulties involved, such as the influence of the enclosing of inflorescences on seed setting, the influence of seasonal variation, of diseases and insect pests, all interfering with comparisons of relative fertility., render the task of evaluating the causes of differential fertility extremely difficult. In a recent publication, Nilsson uses the analysis of variance for assessing the phaenotypical and genotypical influences of variations in self-fertility. When intra-plant variation - the variance of the seed production of single inflorescences - and inter-plant variation, as measured by the variance members of clones, are taken to be the measure of phaenotypical variation, the existence of genotypical differences can be established. Other methods used consist in the correlation of results obtained from the same plant in successive seasons, after transplanting to a different locality; and further in the correlation of self-fertility in successive generations. A genetical method has been used by Williams in measuring the amout of self-fertilization by crossing plants of known genotype possessing genes which revealed the proportion of self-and cross-fertilization.

By all these methods has been shown the existence of hereditary differences in self-compatibility, varying from absolute self-sterility to almost full self-fertility. There is so far no record of accurate inheritance studies on compatibility on the lines of the work on Nicotiana or Veronica; But the evidence of family records seems to show that a comparatively small number of genes is concerned. Nilssen states that "In some inbred families of timothy all plants have a comparatively high degree of self-fertility, in other families all individuals are nearly or completely self-sterile, most families again show a wide segregation... In no instance has a family shown an apparently constant intermediate degree of self-fertility. This indicates that only a few factors, having a great effect, are mainly responsible for the genetical variation in this character." In Red Clover, Williams comes to the conclusion of the existence of at least 15 allelomorphic sterility factors, influencing pollen tube growth on the lines established in Nicotiana.

Pseudo-fertility is frequent in all pasture plants, but, using some of the methods reviewed above, its separation from true fertility should be well possible. But the separation of the action of pseudo-fertility genes (Brieger's gene P in Nicotiana) from sterility genes (S series) should be more difficult in a material the genetical constitution of which is unknown. Williams has led the way in an attempt at identifying sterility groups in Red Clover. It cannot be long before a series of S factors in that plant will be established, and with it a series of test plants possessing the various allelomorphs, which in turn will allow plant breeders to range their material in sterility groups,

In summarizing this paragraph on self-sterility we can state that the analysis of compatibility has revealed its phaenotypical and genotypical foundations; that the latter do not set insuperable limitations to the establishment of absolute sterility or full fertility; that compatibility is a group characteristic, and that any plant can be ranged in one of a number of groups with intra-sterility and inter-fertility; and that to the plant breeder this opens avenues for the full utilisation of the phenomena following inbreeding and outbreeding.

LOSS OF VIGOUR AND HETEROSIS.

When in cross-fertilizing plants Self-fertilization is artificially induced, it is frequently followed by a reduction in the vegetative vigour of the progeny, increasing for several generations till a certain minimum level of vigour is reached, On inter-crossing such inbred non-related strains, normal vigour is re-established. It is further frequently found that even the F_1 Of two non-inbred lines is more vigorous, than either parent, whereas the average vigour in the subsequent generations decreases till again the "inbreeding minimum" is reached. The phenomenon of increased hybrid vigour is called heterosis. An explanation of loss of vigour and heterosis is of vital interest to the plant breeder: in supplying information on the scope and possibilities of inbreeding, that method of mating which is more useful than any other in fixing his types; and in opening up possibilities for the utilization of heterosis, which in one generation may produce improvements which otherwise may be hard to achieve in many years of arduous line breeding.

Just as self-sterility and self-fertility, so loss Of vigour and heterosis have been interpreted in mendelian and in non-mendelian terms. The mendelian explanation sees the advantage of heterosis in the accumulation of advantageous genes, the non-mendelian in heterozygosity in itself, To the plant breeder it is essential to know which is the correct explanation. If the former is right, there is a chance of the heterosis effect being made homozygotic, stabilized; but if the latter fits the case, his only object is to produce the maximum degree of heterozygosity permissible under the circumstances.

The mendelian explanation assumes the existence Of cumulative dominant genes for vigour. The more of these genes an individual possesses, the more vigorous it will ~~be,~~ and in a cross of two forms possessing different vigour genes, the hybrid, combining all dominant genes contributed by the parents, will be more vigorous than either of them. In the subsequent inbred generations homozygosity increases rapidly, and with it the proportion of individuals doubly recessive for some of the vigour genes. The climax of this process homozygosity

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is reached after about 7-8 generations, when almost full heterozygosity is attained. From then on the loss of vigour should be very small. This theory explains satisfactorily the origin of heterosis and loss of vigour. But it requires that segregates should be found of a vigour equal, or even superior, to that of the F_1. To explain their absence or extreme rarity, Jones assumed that the vigour genes are linked and that only by means of crossing-over a high degree of dominance could be established in any but the first hybrid generation. Such crossing-over will only rarely lead to a full complement of dominant genes. On the other hand it explains the origin of the rarely occurring superior individuals in highly inbred, "degenrated", families.

If heterosis is due to genotypical causes, *and* not to the hybrid nature itself, inbreeding in itself cannot be harmful, but only as a mechanism liable to produce unfavourable genotypes. It has been said before that inbreeding differentiates a heterozygotic material. It brings to light recessive characteristics which, in a cross-fertilizing population, would rarely, if ever, appear, such as chlorophyll deficiencies and various other deformities. Such recessive forms can be readily seen and their mode of inheritance is known in many instances. But there also are likely to be recessive inhibitory genes which are detrimental to normal development, although not as effective as e.g. the lethal genes inhibiting chlorophyll development. By "pure-breeding" of lethals, semi-lethals and inhibitory genes, the standard of production is lowered in inbred families. But at the same time inbreeding provides a chance for stabilizing any valuable single lines; We may conclude that according to this theory the effect of inbreeding and outbreeding dependes on the genotype of the material concerned; that in the majority of cases naturally cross-breeding organisms will tend to be favoured by inter-breeding, since inbreeding will produce individuals pure for unfavourable recessives, whereas outbreeding is likely to secure their dominant allelomorphs; but that in a material free from such detrimental recessives inbreeding will lead to stabilized genotypes which may possess full vegetative and generative vigour, although the occurrence of such forms will generally be rare.

Accepting the "interaction-hypothesis" for the effect of quantitative genes recently pronounced by Rasmusson, it would be erroneous to assume a simple additive effect of dominant vigour genes. These genes cannot be supposed to act as independent entities, but as a system of co-ordinated units, whose individual affect will be smaller the larger the number of co-ordinated elements present.

The assumption of the beneficial effect of dominant genes is well in accordance with recent ideas on evolution by gene mutation. It is a general fact that wild types largely possess dominant allelomorphs, whereas mutations arising in them - most frequently of a harmful nature - are mostly recessive. Obviously harmful dominant genes would be eliminated very rapidly in nature, whereas recessives of a similar effect may be preserved for very long periods, provided the normal allelomorph is fully dominant. R. A. Fisher suggested that this prevailing dominance in wild types has been gradually developed by the acquisition of modifying genes which determine the full dominance of the normal over the harmful recessive allelomorph.

The "heterosis" theory, as distinct from the "dominance" theory outlined above, explains heterosis and loss of vigour by the relative physiological merits of heterozygosity and homozygosity. The former is assumed to produce a stimulating effect, and the latter the opposite. Whereas for the general phenomena following hybridization and subsequent inbreeding the theory is acceptable, it does not fit the case, where individual problems are concerned: E. g., hybridization of unrelated forms does not always produce a superior hybrid, Further, homozygotic types may be more vigorous than corresponding heterozygotes.

This theory has received recent support from the results obtained by Engledow and Pal in a study on hybrid vigour in wheat. Mainly owing to a marked, though small, difference in hybrid vigour exhibited in reciprocal crosses, the authors contend that a factorial explanation is not satisfactory. The main effect of heterosis was seen in the earlier stages of plant development. The authors observe therefore. that "it strikingly resembled the early "stimulus" observed to result from some "seed dressings" (fungicidal) used for cereals which also loses its effect long before the plants reach maturity. It seems not unreasonable to suggest therefore that hybrid vigour, as a phenomenon of juvenile life is, at any rate in part, of a nature of a temporary stimulus set up by the act, or consequences, of causing unlike gametes to unite." Although the case in question seems to require further investigation before the general conclusion may be accepted, it seems possible to me that there may be several mechanisms producing similar effects. In reviewing the literature on hybrid vigour Engledow and Pal found that it appeared most frequently and marked in plants being (a) diploid, (b) naturally out-pollinating and (c) endospermic in seed, although "the significance of this association ..... with hybrid vigour cannot yet be judged." I think it must be conceded that in those organisms in which heterosis is most marked, i. e., with few exceptions in plants satisfying these three conditions, the dominance theory seems to give a satisfactory explanation of the facts. Wheat on the other hand is polyploid and self-pollinating, and the small amount of hybrid vigour observed may be due to a different mechanism from that shown by typical heterosis organisms.

Altogether the incidence of polyploidy seems to have a noticeable influence on sterility as well as on loss or increase of vigour. Polyploids frequently are more self-compatible, and exhibit loss of vigour and heterosis to a lesser degree than corresponding diploids. This has been demonstrated in diploid and hexaploid Phleum pratense, the latter being more self-compatible than the former. This phenomenon is doubtless due to the larger number of compatibility genes, and their greater chance of recombination, in the polyploid form. Production of polyploid varieties should therefore be a means for increasing both vigour and self-compatibility.

An accurate measurement of the effect of inbreeding in pasture plants encounters considerable technical difficulties, as has been fully discussed by Nilsson in a recent paper. He was, however, able to prove that neither the influence of the isolating process, nor of selfing in itself, was as powerful in determining the vigour of the progency as the factorial constitution of the mother plant.

It has been frequently stated that inbreeding causes a decrease in fertility, becoming more intense in successive generations. Nilsson's investigations show that this is not generally the case, but that different types occur exemplifying a wide segregation for fertility amongst inbred families. Similarly, Williams reported that in Red Clover there is "no reduction in the degree of fertility ... as the result of inbreeding."

The low germination in some inbred families in Lolium perenne is considered to be due to lethal factors. In Timothy, on the other hand, germination was higher in inbred than in outbred families. Chlorophyll defects and various kinds of lethals and malformations are common in practically all inbred pasture plants, as they are in maize, and even in self-fertilizing plants such as barley. Their occurrence, just as that of plants of low winter hardiness found by Nilsson in inbred material, is doubtless due to segregation, Using the height of the culm as a measure for vigour, Nilsson found remarkable differences between various families in their reaction to inbreeding. Whereas in all plants investigated - except in highly self-fertile species - the large majority of families was more vigorous after open-pollination than after self-fertilization of the mother plant, there are a number of lines in all crops where the differences, positive and negative, are not statistically significant, and in three lines of Timothy the inbred family is significantly more vigorous than the out-pollinated one.. Nilsson concludes that this is entirely due to the segregation of vigour 'genes. The existence of positive correlations between various plant characters of essential importance to the development of the organism, such as panicle length, tillering and fertility, with height, led Nilsson to the assumption of "vitality factors" of pleiotropic effect. The different behaviour after inbreeding of different species, strains or lines is due to differences in genetic constitution, chiefly with respect to these vitality factors. It may be recalled that in Cocksfoot Stapledon made similar observations on vegetative vigour as a hereditary characteristic showing segregation on inbreeding,,

We have now arrived at the point where conclusions of a general nature can be drawn on the applicability of these fundamental principles to the breeding of pasture plants. Self-fertility, in the first instance, having been proved to be genotypically founded, should be accessible to combination with economic characters , provided that self-fertility were a desirable characteristic, and that linkage barriers are not insuperable. Self-fertility will be desirable only in an attempt to fix a type with a maximum dosis of vigour genes - apart from other desirable economic characters, But from 'past experience as well as from the theoretical considerations outlined above, it is more than likely that such individuals will be extremely rare in ordinary F_2's F_3's etc. In America the attempt has been made to produce such individuals by a system of back-crossing and selection, using both parents in separate lines which are ultimately intercrossed. This should lead to a high degree of homozygosity for dominant genes contributed by both parents. This method might be applicable to pasture plants in a long-range breeding scheme. Obviously it would be advantageous if the ultimate product possessed a high degree of. self-fertility.

But even in less ambitious schemes. self-fertility as a line characteristic is of vital importance where inbreeding, or mating between close relatives, is contemplated even as a temporary measure. It seems essential to me that a maximum amount of information should be collected on the degree of inducible Self-fertility in important breeding material.

What has been said about information on self-fertility is equally applicable to self-sterility. This characteristic may in some circumstances be more important than the former. If hybrid vigour is to be attained by the intercrossing of lines, self-fertilization is strictly undesirable. This is one further reason why fertility data are important. And finally, the problem of cross-' ferility and cross-sterility will some time have to be tackled. It is conceivable that compound strains may be built up producing nsther Seed with a maximum of hybrid vigour. How much would the selection of components be facilitated by the exact knowledge of their sterility genes! It occurs to me that this type of work essentially calls for co-operation on a large scale, There is no reason why material of known constitution, once it was found, should not be utilized by workers all the world over, for constructive breeding as well as for classification.

It is obvious that at present there still is a large field for simpler methods. But the time seems to be near when a further step will call for material and information, and we should be building for the future in- attempting to provide both.