STUDIES ON SELF-STERILITY. IV. SELECTIVE FERTILIZATION

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INTRODUCTION

Selective fertilization has been evoked many times as a means of accounting for peculiar or unusual breeding results. CASTLE'S (1903) original theory of sex-determination, and CUÉNOT'S (1908) interpretation of the non-appearance of homozygous yellow mice, are examples. Fortunately, it has always been possible to explain matters without retaining the hypothesis; in many cases, in fact, direct proof has been available that selective fertilization does not occur. Nevertheless, selective fertilization as a contingency has remained a sort of nightmare to investigators in genetics. Such antipathy is not unnatural, but one must have in mind the changes which have taken place in the subject during the last decade, to understand clearly the reason.

MENDEL'S discoveries, the Laws of Segregation and of Recombination, made heredity enticingly simple. All extensions, additions and exceptions have tended toward complexity. In this genetics has but repeated the history of chemistry and physics, yet it is to be expected, perhaps, that any suggested change in genetic conceptions savoring of increased complexity should find favor slowly. And selective fertilization is a tenet which would increase the difficulties of the subject a hundred-fold.

The outgrowth of Mendelism has been a theory of inheritance founded on the conception of specific character determiners, genes, located in the chromatin. In the sense that the central problem of heredity is clearly

one of chromosome, or at least of chromatin, distribution, the modern generalization has a simple grandeur not found in early Mendelism; but this simplicity is quite delusive, as a short consideration shows.

The conception of the gene is unquestionably the foundation of genetics. Students of heredity have submitted good evidence that *characters* are the product of many relatively *stable* genes which have a real basis in the germplasm, and that each of these genes may be the cause of various effects in different parts of the organism. They have shown that while the effects of a particular gene may not be wholly the same under different environmental conditions, nevertheless neither changes in the factors of environment nor association in particular combinations in the germplasm serves to change their individuality or constitution with a *significant* frequency.

Heredity, then, is the distribution of genes, and the genes have been located definitely in the chromosomes. Fortunately, chromosome distribution has been standardized in a remarkable manner in the majority of plants and animals; hence, the greater part of the phenomena found in breeding experiments may be described by a comparatively few simple mathematical formulae. It is to this orderly chromosome distribution that one must impute the utility of the Mendelian nomenclature, for to it in large measure is due the regularity with which certain ratios recur. There are irregularities in chromosome distribution, it is true. They have even furnished some of the critical tests of the modern theory of heredity taken as a whole. But because they curtail the practical value of the theory through limiting the possibilities of prediction, it is well that they are rare.

The standard chromosome mechanism for distribution of genes is that in which homologous chromosomes mate at synapsis, and homologous genes, *one* from either parent, pass by chance to either pole of the mitotic figure, in the formation of the mature gametes. The chromosomes may separate without having exchanged genes, presumably; or, genes may be exchanged. Just how this interchange occurs is not wholly clear. MORGAN has assumed that the genes have a linear arrangement, and that there must be transverse breaks in the chromosomes. CASTLE (1918) believes the arrangement is not linear, and that breaks may occur in many ways. It is possible that neither assumption is correct. The writer has felt for some time that possibly the genes are arranged spatially in a manner somewhat analogous to that assumed by chemists for organic molecules, though perhaps it might be better to say in a manner an-

alogous to certain crystals, for there certainly is no evidence that the genes are radicles belonging to single molecules. But the point is that with a spatial arrangement similar to that assumed for the radicles of molecules, with the homologous chromosomes mirror images of each other, with homologous genes interchanging by a definite mechanism, a more delicate system of action is possible than with mere chromosome breaks.

However this may be, the hinge on which the usefulness of this whole scheme turns is that *the genes pass to either daughter cell by chance*, and that the gametes thus formed *mate by chance*.

Even when such inheritance obtains, selective elimination of both gametes and zygotes is somewhat common, and causes rather chaotic conditions wherever it occurs. For example, the difficulties which characterize all endeavor to analyze inheritance in the *Oenotheras* are probably due in large measure to this cause. The additional difficulties which would arise should it be found that there is *selection of genes* in gamete formation, and *selection of gametes* at fertilization are so great as to be hardly imaginable.

DISCUSSION OF THE PROBLEM

Particularly suitable material with which to test the second possibility is found in those plants which are self-sterile. Since the direct cause of self-sterility is the slowness of growth of *self* pollen tubes as compared with *cross* pollen tubes, it would seem as if selective fertilization would have a better opportunity to manifest itself under such circumstances than under those which obtain in self-fertile plants and in animals.

Experiments with the self-sterile species *Nicotiana Forgetiana*, *N. alata* and *N. angustifolia* have shown that in self-pollinations and in incompatible cross-pollinations the pollen grains germinate as well as in compatible cross-pollinations. No differences are to be found between the two types either as to the percentage of grains germinating, the length of time required for germination, or the size of the tubes after germination, provided pollen tubes of the same length are measured. Pollen tubes produced after self-pollination or after incompatible cross-pollination grow so steadily that length plotted against time is a straight line; but pollen tubes produced after a compatible cross grow at such a constantly increasing rate that the growth curve resembles that of an autocatalytic reaction. As the flowering season is about to come to an end, more rapid pollen-tube growth occurs after a self-pollination or an in-

compatible cross-pollination, though there is little evidence of the accelerated growth characteristic of compatible combinations. The pollen tubes grow more rapidly, but the curve by its constant velocity still resembles the curve of a "normal" self-pollination.

These facts are the basis of our problem, and naturally they suggest the possibility of selective fertilization. Part of the work reported in the first of these studies (EAST and PARK 1917) was done upon a cross between *N. Forgetiana* and *N. alata*. The segregating generations naturally contained numerous individuals heterozygous for a large number of hereditary factors. There were differences in height of plant, size of leaf, color of flower, and size of flower, differences which could hardly be interpreted as the result of less than twenty or thirty determiners unless a great many of the variations shown in different organs were due to the activity of a single gene. Similar hereditary differences were marked even in the so-called pure species. This being true, it is important to know whether pollen tubes whose nuclei carry certain determiners grow faster than those which carry other determiners.

POLLEN-TUBE FREQUENCY DISTRIBUTIONS

One method which throws some light on the probability of selective fertilization is that of studying the frequency distribution of the pollen tubes after pollination. When applications of pollen are made, and the pistils prepared, sectioned and stained at varying periods of time after pollination, similar results are obtained no matter what the type of combination has been. In table 1, for example, a few frequency distributions of pollen-tubes after self-pollinating self-sterile plants during the height of the flowering season, are given. In general they are minus skew, and show that the greater number of pollen tubes are grouped at points from 3.5 mm to 7.5 mm from the end of the stigma at the expiration of from 5 to 7 days after pollination. A number of tubes have pushed out ahead of the majority, and a great many pollen grains—from 5 to 25 percent—have not germinated at all.

Distribution of pollen tubes in sections of pistils from self-sterile plants which had been pollinated at the end of the flowering season, show practically the same thing (table 2). The pollen tubes have reached distances comparable to those shown in table 1 in a shorter period of time, but otherwise no marked difference can be seen.

These two tables are presented merely for comparison with tables 3 and 4.

TABLE 1

Frequency distribution of pollen tubes after self-pollinating self-sterile plants during the height of the flowering season.

Source of data	Distance from the stigma in millimeters													
	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
I section after 5 days.....	2	20	25	20	14	6	5	2						
I section after 6 days.....		1	43	30	30	20	13	7	7	5	2	2	1	
I section after 6 days.....	2	2	50	41	40	16	17	6	5	2	3	1	1	
I section after 7 days.....					4	20	24	18	9	7	2	2	1	
I section after 7 days.....			1	6	20	25	21	16	16	8	6	2	1	1

TABLE 2

Frequency distribution of pollen tubes after self-pollinating self-sterile plants at the end of the flowering season

Source of data	Distance from the stigma in millimeters													
	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
I section after 3 days.....	3	3	20	30	16	6	2	1	1	1				
I section after 3 days.....		6	30	21	20	18	6	2	2	1				
I section after 4 days.....		1	6	20	28	16	8	4	5	2	1			
I section after 4 days.....			2	6	18	25	26	24	20	16	8	2	1	
I section after 4 days.....						2	18	20	18	8	6	4	3	1

In table 3 some distributions of pollen tubes from the F_2 generation of a cross between *N. Forgetiana* and *N. alata* are given. The cross is compatible, and since the individuals are unquestionably heterozygous in a large number of factors, they should show a marked tendency to vary if there is selective fertilization. The frequency distributions shown in table 4, on the other hand, where *sib* matings for three generations ought

TABLE 3

Frequency distribution of pollen tubes after cross-pollinating compatible plants of F_2 generation *N. Forgetiana* $\times$ *N. alata*.

Source of data	Distance from the stigma in millimeters											
	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5
I section after 2 days.....	3	16	26	28	24	21	16	12	8	2	1	1
I section after 2 days.....	6	20	21	16	8	3	1					
I section after 2 days.....			1	16	20	18	16	8	3	3	1	
I section after 3 days.....				1	16	15	14	8	4	2	2	2
I section after 3 days.....			1	6	40	28	20	16	3	1	1	

TABLE 4

Frequency distribution of pollen tubes after cross-pollinating compatible plants of F_2 generation (sib matings) *N. Forgetiana* $\times$ *N. alata*

Source of data	Distance from the stigma in millimeters											
	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5
I section after 2 days.....		6	18	24	20	18	7	2	4	2		
I section after 2 days.....			2	36	30	20	18	7	6	5		1
I section after 3 days.....			1	16	26	21	17	5	5	2	1	
I section after 3 days.....				2	24	21	18	3	8	1	2	
I section after 3 days.....					1	16	26	21	20	14	10	4

to have brought about a considerable degree of homozygosis, should be less variable. As a matter of fact, however, there seems to be no significant difference in the two cases.

There is no evidence that variability in gametic constitution is the cause of variability in rate of pollen-tube growth. In fact, there is no positive proof that there is a measurable variability in pollen-tube growth.

In both of these types of pollination and in all similar cases examined, percentages of ungerminated pollen grains comparable to those determined for incompatible matings were found. The actual percentages have little meaning, for ungerminated pollen grains are loosely held by the stigmas and the correct number of grains which do not germinate is not likely to be obtained. But the fact that a considerable percentage of grains which contain protoplasm and in every respect seem to be normal, remain as long as 6 days without germinating, leads one to believe that difference in the rate of germination is largely responsible for the varied length of the pollen tubes measured. The pollen grains may differ among themselves in the thickness of their walls or the composition of the protoplasm outside the nuclei, thus accounting in some measure for rapidity of germination, without it being necessary to assume gametic differentiation as a cause. Furthermore the entire series of results on the behavior of self-sterile plants reported in the first paper of this series (EAST and PARK 1917), makes it unlikely that differences in gametic composition show themselves in any way *before fertilization*. The factorial composition of the mother plant controls the behavior of self-sterile plants, and all the pollen grains of a single plant may be taken to have the same factorial composition as far as any functions to be performed *before* fertilization are concerned.

It is not to be supposed that the variability in length of pollen tube shown in tables 3 and 4 really represents the difference of time at fertilization. In compatible matings the pollen tubes grow faster and faster so that the variability shown in a frequency distribution of pollen tubes determined at 1 day or 2 days after pollination may be quite different at a later date. It has not been found possible to obtain satisfactory measurements of pollen tubes as they approach the micropyles, but it may be assumed that at this time the rate of growth is so fast that practically all of the ovules are fertilized within a few hours. Selective fertilization is hardly probable therefore for this additional reason.

INFLUENCE OF THE GROWTH OF COMPATIBLE POLLEN TUBES UPON INCOMPATIBLE POLLEN TUBES

In interpreting the results of our experiments on pollen-tube growth (EAST and PARK 1918), it was assumed that after a compatible cross substances are secreted in the pistil which accelerate the elongation of the tube, and that the immediate cause of this secretion is a catalyser which the pollen-tube nucleus is able to produce because the hereditary consti-

tution of the plant producing it is different from that of the plant on which it is placed. Superficial consideration might lead one to suppose that if this were true, incompatible pollen tubes would be accelerated by the growth of compatible pollen tubes if a mixture of the two kinds of pollen were placed on the stigma. Second thought, however, shows that this is probably not the case. Plant enzymes are colloids having large molecules, hence they do not pass freely through cell membranes. Their actions are largely local; where they do not seem to be local, the direct cause of the reaction is more likely to be a crystalloid produced by action of the colloid.

The writer has been able to devise no experiment to measure absolutely such possible stimulation, but two experiments have shown that when mixtures of compatible and incompatible pollen are applied to a single stigma, only the compatible pollen produces seed.

In the first experiment a number of pistils were pollinated with a definite number of compatible pollen grains. The work was done under a binocular, and the count is thought to be accurate within an experimental error of ± 2 grains. The pistils were then carefully covered with incompatible pollen. Eight capsules matured with the results shown in table 5.

TABLE 5
*The effect of compatible pollen on the growth of
incompatible pollen tubes.*

Pistil No.	Number of compatible pollen grains	Number of seeds produced
1	51	46
2	48	42
3	50	41
4	62	49
5	32	23
6	67	58
7	61	54
8	46	40

The indications from this experiment are that no incompatible pollen tubes contributed to the production of the seeds obtained; but of course it is impossible to maintain that these tubes were not accelerated in their growth to some degree.

In the second experiment, a more critical test of the matter was made. Three pistils of a white-flowered self-sterile plant coming from a line of

plants homozygous for this color were selfed. Five or six hours after these plants were covered with pollen from a self-sterile family bearing red flowers. Capsules full of seed were obtained. If these seeds were produced by the compatible pollen only, the resulting progeny should be red-flowered for red is dominant; if incompatible pollen has functioned, white-flowered plants should be obtained. Three hundred plants have been grown with *not a single* white-flowered individual.

SUMMARY AND DISCUSSION

The experiments described in this paper were designed to test the possibility of selective fertilization occurring in self-sterile *Nicotianas*, it being assumed that from the nature of the material the phenomenon might here be possible. (1) Comparisons were made between the pollen-tube frequency distributions of highly heterozygous and of comparatively homozygous plants. (2) The influence of compatible matings on incompatible matings was investigated. In neither case was there any indication of selective fertilization.

Though it is impossible to prove a negative, there is so much circumstantial evidence against selection both in the formation of gametes and zygotes, the probability that it ever occurs is very remote. In the first place gametes are formed in many animals and plants, particularly in species crosses, which can never function. If the mechanism of gamete formation were such as to make it necessary to assume a selection of genes, a low frequency of non-functional gametes would be expected. Similarly zygotes are produced in the numbers to be expected by chance mating of gametes, even though these zygotes have no possibility of passing through a complete life cycle. There are two cases in mice, eight in *Drosophila*, and four in plants where the evidence of lethal factors is too complete to be disregarded. In reality there are probably hundreds of such instances in plants and animals which have been investigated during recent years that ought to be interpreted in the same manner.

Again, pollen grains show no tendency to behave as if the genes which they carry function before fertilization. It will be recalled that BATESON (1909) found pollen shape and color in the sweet pea to be inherited as a maternal character. The writer (EAST 1916) has corroborated this discovery for color of *Nicotiana* pollen. It may be claimed, however, that these facts are just what is to be expected because of the morphogenesis of the outer characters of the pollen grain. This is true; but

the criticism does not apply to the phenomena found in the behavior of self-sterile plants in cross matings where cross-sterility of groups of plants exists *presumably because of genes possessed by the mother plants*. In fact the only activity shown by a male gametophyte which seems to be due to the factors it is carrying over into the next generation, is a lack of any activity. In BELLING'S (1914) work on the velvet bean, he found 50 percent of the F_1 pollen was abortive in a certain cross. It appears then that in this instance the presence or absence of a gene of the generation which would ordinarily function after fertilization, has caused the pollen grain to abort. This lack of ability to function does not necessarily mean the actual activity of the genes of this generation however; the machine has simply remained uncompleted, so to speak. For this reason, there seems to be no wisdom in even suspecting selective fertilization; unless mixtures of pollen (or spermatozoa even) from different individuals should be used. If pollen grains from a single plant are alike as far as their activities before fertilization are concerned, there is no basis for selection.

May we not extend this conception to animals for the present and accept as a fundamental genetic hypothesis the tenet of chance segregation in the germ cells and chance mating of these germ cells?

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