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Selection in the Production of the Ever-sporting Stocks.

BY

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With one Diagram in the Text.

I. *Winge's Theory of Doubleness.*

THE problem of doubleness in stocks, which had been the subject of genetic work at Cambridge from almost the beginning of the century, has been recently cleared up by Winge (O. Winge, 1931, 'The inheritance of double flowers and other characters in *Matthiola'*, Zeitschrift für Züchtung, Reihe a Pflanzenzüchtung, xvii. 118-35), by means of extensive experiments of his own, which he finds confirmed by certain unexplained exceptional plants reported by Miss E. R. Saunders as early as 1911.

On Winge's theory the double is a recessive mutant which, since the double flowers are sterile, both as pollen and ovule parent, acts as a lethal. In the ever-sporting races it is balanced by a closely linked pollen lethal. The ever-sporting singles, from which the doubles are derived generation after generation, are thus heterozygous for two closely linked lethals carried in opposite chromosomes. Apart from rare recombinations, the pollen all contains the gene for doubleness, while the ovules are of two kinds, one containing the gene for doubleness, and the other the pollen lethal. Consequently there are produced in each generation nearly half doubles, free from the pollen lethal, and nearly half singles carrying this lethal like their parents. The pollen lethal acts, not only on the gamete, but has also a debilitating effect on the heterozygote; the singles of ever-sporting lines are for this reason relatively weakly plants, compared with the doubles of the same lines, and with singles obtained by out-crossing. Their frequency also shows regularly some slight deficiency compared with the doubles in the same families. This inequality in the numbers surviving to be classified led, for many years, to an elaboration of hypotheses aimed at explaining, by the interaction of two or more factors, the aberrant and irregular ratios observed.

In addition to the offspring produced, as explained above, by non-crossover gametes, there should, on Winge's hypothesis, be formed a small proportion of normal pollen, which, with the two common types of ovule, will produce two types of single plant bearing either the pollen lethal, or the recessive gene for doubleness, but not both. The second type will also be produced from cross-over ovules. From the progeny of such plants both of these recessive factors will be steadily and automatically eliminated. The pollen lethal could of course only survive in the female line, and even here will be at a disadvantage owing to its debilitating effect. The doubling factor can survive both in pollen and ovules, but since the double plants themselves must be without progeny, the power of throwing doubles could only be retained in the strain by planting different progenies separately, and, in each generation, taking seed only from those singles which had appeared in the same families as doubles. Since this precaution is not needed in the ever-sporting strains of stock, it would certainly not have been taken ; and the progeny descended from such cross-over plants, throwing, as long as it was retained, an increasing proportion of singles, would be certainly rejected sooner or later as contaminated seed.

One cross-over plant, from which the pollen lethal had been eliminated, was fortunately used as a parent by Winge, who raised 186 plants from it by self-fertilization; of these 139 were single, and 47 double. Evidently, it differed sharply from the sister plants which gave, as usual, a slight excess of doubles. It was also evidently much more fertile than they were. Only a quarter of the offspring are doubles, and we should therefore expect a second quarter to be pure-breeding singles. Actually, two of the single offspring bred from gave respectively progenies of 945 and 314 plants, all single, while others gave large progenies very closely in the ratio 3:1.

In addition to this well-established case of his own, Winge points out that a probably similar family was reported by E. R. Saunders (1911), ('Further experiments on the inheritance of "doubleness" and other characters in stocks', Journal of Genetics, i. 303-76). Of the 87 self-bred families of the Glabrous-red race (Table III, p. 372), the 86th has 23 singles and 8 doubles, numbers strongly suggestive of a 3:1 ratio, and very aberrant from the excess of doubles usually found, especially in the smaller families. Unfortunately, this particular family was not further propagated, so that direct proof of its nature was not obtained. Indeed, it would seem that the unusual ratio which it shows must have been entirely overlooked, since the summary of the results with this strain (p. 307) commences with the words 'Thus every attempt to breed out the doubles proved unsuccessful', a conclusion which must be regarded as most unfortunate in view of the probability that doubleness would have been promptly 'bred out' had the first family which showed a decided deficiency of doubles been used for the purpose. That Winge is undoubtedly right in regarding the usual excess of doubles as wholly due to differential viability, and this particular family as exceptional will be made clear in the following section.

II. *A Graphical Method of Examining Frequency Ratios,*

The fact, which for many years misled geneticists with regard to the problem of double stocks, was that in the ever-sporting strains, the

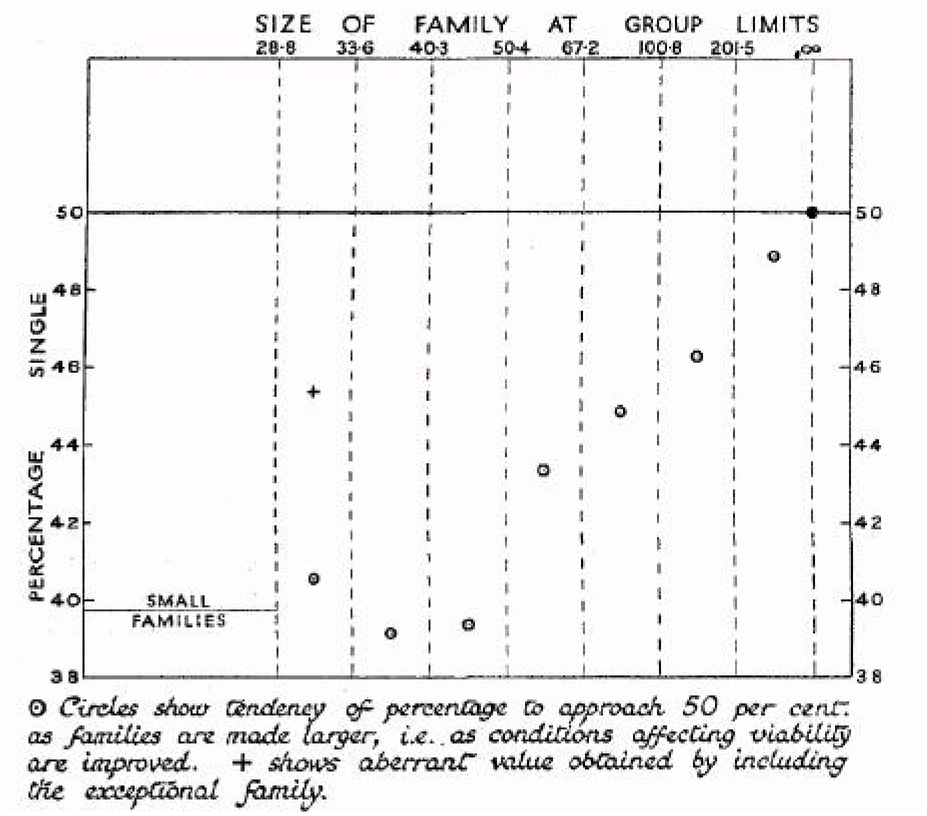

Diagram showing proportion of singles according to the size of family.

numbers of single and double plants surviving to be classified, were not equal. A significant and fairly regular excess of double plants was constantly observed. Such departure from simple ratios may be due to unequal viability, or equally it may be suspected that a more complex genetic hypothesis is required. A simple graphical device, which, with appropriate data, will readily indicate, on internal evidence, that the whole of the discrepancy must be ascribed to unequal viability, may therefore be of use in other cases. Using the group of 87 Glabrous-red families, which were published by Miss E. R. Saunders in 1911, it will be seen that had any such device been applied to examine the data at this date, no reasonable doubt would have remained that the ratio of singles to doubles was genetically a 1 : 1 ratio, in spite of the persistent and significant excess of doubles observed in most families.

The method consists in plotting the percentage of singles in families of different sizes, The boundaries between the successive size-classes are chosen to be in harmonic progression, with the upper boundary of the highest class at infinity. The successive boundaries are thus found by dividing some chosen number by 0, 2, 4, 6, ... Since it is desirable, to avoid ambiguities, that these boundaries shall not be whole numbers, it is as well to choose, as the fundamental number, upon which the sequence is to be based, one that is odd. We require in addition that it shall not be so much as twice as great as the largest family, which in this case has 249 members. Further, since we shall use the same diagram to exhibit the aberrant character of a particular family of 31 members, we can make sure that this family shall be centrally placed in the group in which it falls by choosing a multiple of 31. We shall choose 31 x 13, or 403, and use the group limits found by dividing 403 by 2, 4, 6, 8, 10, 12, and 14; the values of which to one decimal place are given at the top of the diagram. All families of more than 28 members can be placed in one of these seven classes ; the total numbers of singles and doubles obtained from each class can be enumerated, and the percentage of single plants plotted, as in the diagram. The horizontal line on the left of the diagram indicates the percentage of singles in families of less than 39 individuals, which have not been further subdivided. The Table below shows the totals in the different classes:

Summary of 87 Glabrous-red Families ; Data Plotted in Diagram.

Size of family.	Single.	Double.	Total.	Percentage Single.
Over 201	239	250	489	48·88
101 to 201	433	503	936	46·26
68 to 100	261	321	582	44·85
51 to 67	180	235	415	43·37
41 to 50	102	157	259	39·38
34 to 40	130	202	332	39·16
29 to 33 (all families)	(98)	(118)	(216)	(45·37)
29 to 33 (Omitting 1 family)	75	110	185	40·54
Less than 29	200	303	503	39·76

It will be seen that the first four classes, where the percentages are based on the counts of more than 400 plants, point unmistakably to 50 per cent. (the black spot on the diagram) as the limiting value to which the percentage tends as the causes of mortality are more and more thoroughly removed. It has happened in this material that size of family has provided a sufficiently good basis for estimating the favourableness of the conditions in which the plants were reared, and, by extrapolation, for inferring the genetic ratio appropriate to ideal conditions. In other cases it might be necessary to use germination percentage rather than size of family as a basis for classification. The method merely brings to our notice such indications, whether they are only slight, or, as in this case, so strong as to be decisive, as the data happen to contain. That the internal evidence should be so decisive, is a remarkable tribute to the intrinsic excellence of the data.

We may now consider the exceptional family with 33 single against 8 double plants. A glance at the diagram shows that the expectation for families of this size is about 40 per cent. singles, or roughly, 12 single plants to 19 double. The existence of families with a large excess of doubles (e.g. 9 singles to 23 doubles in family 6) would therefore be not intrinsically improbable, and their occurrence would be no reason for disregarding the family with an exceptional excess of singles. If any doubt remained on this point, or if this particular family had been overlooked, the percentage of singles obtained for this size-class, and represented by a cross on the diagram, would have shown that the inclusion of this family had been sufficient to disturb the average of its class, to an extent which the regularity of the other points shows at once to be inadmissible.

The same diagrammatic examination of the frequency ratio, would therefore, in this case, have provided decisive evidence that the discrepancy from a 1: 1 ratio was due to differences of viability only ; and, at the same time have drawn attention to the really exceptional character of family 86.

III. *Modification of Linkage Intensity.*

In discussing the origin of the ever-sporting type Winge (p. 131) naturally suggests that the first step was the occurrence of the mutation for double flower, which can at first only have been perpetuated by the continual selection by the growers. Selecting singles from families, or seed batches, which threw the highest proportion of doubles they would have immediately seized upon and brought into general prevalence the ever-sporting type of single, as soon as the pollen lethal had occurred in the single-bearing chromosome of a heterozygote. Winge quotes Miss Saunder's conclusion that the ever-sporting combination must have come into existence before the end of the seventeenth century, possibly not long after the double-flowered type was first known, but he does not call attention to the interesting process of selection which, according to his theory, must have taken place during the 200 or 250 years, since the pollen lethal was introduced. During this period the ever-sporting types of stock must have retained, generation after generation, only the progeny of non-cross-over gametes, for the cross-overs, as we have seen, would rapidly degenerate into singleness, and, though they might occasionally form new single varieties the effect is the same as though they were necessarily discarded, for their germ plasm will not again be introduced into the ever-sporting strain. Within such strains, therefore, selection must constantly have favoured closer linkage, and this explains what would otherwise have to be regarded as a somewhat remarkable coincidence, namely, that the lethal needed to balance the doubleness should have occurred exactly where the gardener wanted it, or at least within about 1 cross-over unit of the gene for doubleness. Recognizing that cross-overs have been rigorously eliminated we might suppose, on the other hand, that the cross-over percentage originally was as high as 10, perhaps, or 20 per cent, and that it has been lowered progressively by the selective elimination of those strains in which recombination took place most freely.

The conclusion that the extremely close linkage observed is due to the recent action of relatively intense selection is supported by three further facts:

(i) Although two of the races used by Miss Saunders, the Glabrous-red and the Sulphur-white, were composed entirely of ever-sporting individuals, yet the Glabrous-white and Cream plants were mixed in type, some breeding true to singleness, others throwing three singles to one double, and others again throwing an excess of doubles like other ever-sporting plants. On Winge's hypothesis such a mixture would inevitably arise sooner or later in the propagation of pure lines of seed, without any outside contamination, simply by the occasional occurrence of cross-overs, and the elimination of doubleness in their descendants. The frequency of such impure batches of seed, suggests that in most strains the frequency of recombination is higher than in the reliable ever-sporting strain tested by Winge.

(ii) An even more striking example of the continued existence of occasional individuals with relatively high frequencies of recombination is afforded by plant K of Miss Saunders's Cream line. Progenies were grown from 49 single-flowered offspring of this plant and of these, though certainly the majority were true to the ever-sporting type, at least five showed good 3: 1 segregations, and five more gave only singles. Winge says ' Miss Saunders is herself at a loss for an explanation of the phenomenon, and my theory does not give any satisfactory explanation either. For it would imply a rather frequent crossing-over, and this is contrary to the findings in other experiments. I am rather inclined to think that we are here dealing with an experimental error, but, of course, it is not very satisfactory to try to explain the results of other investigators by ascribing them to some slip in the technique'.

Now if the close linkage observed in some reliable ever-sporting strains is due to the selection of the last 200 years it is by no means improbable that other less reliable strains have persisted in which the frequency of crossing-over is considerably higher. In such strains we should expect most frequently to come across such an apparent ' mixture of seed ' as is noticed by Miss Saunders, and also such plants as K of the Cream strain, which, while still of the balanced-lethal constitution, have so high a rate of crossing-over, that, if they are largely used as parents, their offspring will rapidly show signs of such mixture. There is thus no reason to suspect a slip in technique and, indeed, the plant may be cited as a remarkable confirmation of one of the most interesting consequences of Winge's theory.

(iii) The effect of selection in favour of close linkage between two loci must be to diminish principally the crossing-over between them ; but also, presumably in less degree, in the two adjacent segments of the same chromosome. Its observational effect will therefore be to shorten greatly in the intervening segment, and to some extent elsewhere, the map length of the chromosome in question, and so to increase the probability of other close linkages found in the same chromosome. It is therefore relevant to the view that the close linkage between the factor for doubleness and the pollen lethal found with it, in ever-sporting strains, has been produced by the selection inherent in the propagation of these strains, that these two factors are both closely linked with the factor for yellow plastids.

SUMMARY.

1. An outline of Winge's theory of doubleness in stocks is given, and of its implications.

2. A simple method of diagrammatic representation applied to Miss Saunders's data of 1911, shows both that the observed excess of doubles is due solely to their greater viability, and that one family there reported was exceptional in giving only one quarter doubles, as should the progeny of a plant freed from the pollen lethal.

3. The close linkage between the pollen lethal and the factor for doubleness is due to selection acting automatically in the propagation of the ever-sporting lines, which has thus built up the ever-sporting character.