

THE GENETIC BASIS OF STERILITY IN SOME *DROSOPHILA* HYBRIDS

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(With Two Text-figures)

Dobzhansky & Boche (1933) showed that within the species of *Drosophila pseudo-obscura* and *D. persimilis* there are strains of differing 'strength' in regard to the opposite species, the strongest (by definition) being those strains which, in the species cross, produce hybrid males with the greatest degree of abnormality in the testes. Crosses between 'weak' strains of both species, on the other hand, may produce hybrid males in the testes of which may be found complete chromosome pairing at meiosis. Further observations suggested to the authors that the genes determining strength or weakness in the sense used were probably identical with the genes responsible for sterility in the hybrid males. The theoretical conclusion was reached that within a species exist 'the genetic elements from which a mechanism causing hybrid sterility may, theoretically, be built'. In a series of experiments involving six different marked strains of *D. pseudo-obscura*, the present author showed (Lamy, 1943) that hybrid females which had had as the *pseudo-obscura* parent a male or female of one of several marked strains were completely sterile when backcrossed to the parental marked strain. The data showed that the genotype as a whole, and not only the marker genes, was responsible for the sterility, and that the age of the marked strain used seemed to be a determining factor in the degree of sterility shown by the hybrid; the older the strain, the greater the sterility. If the flies of the marked strains were first outcrossed to a wild-type strain of their own species, it was found that hybrid females formed from this mixed *pseudo-obscura* genotype and the wild-type *persimilis*, were partially fertile even when backcrossed to males of the old marked stocks. Yet the only genetic difference between these hybrids and those which showed complete sterility in the backcross to the marked stock was that a portion of their *pseudo-obscura* autosomes was derived from a wild-type stock, while all of the *pseudo-obscura* autosomes of the sterile hybrids were derived from the old marked stocks.

In order to explain this situation it was postulated that some type of genic deterioration had occurred in the old marked stocks (and not in the wild-type ones of equal age), the effect of which was to exaggerate the specific differences between the *pseudo-obscura* and *persimilis* genotypes, in such a way that mixtures of the two types of chromosomes could not result in viable offspring, if the males used in the backcrosses contained only the 'degenerated' chromatin of the old marked stocks. Some admixture of wild-type chromatin in the cross, however, increased the chances of obtaining such viable offspring in the backcross.

There was, however, one possible objection to this hypothesis. If the sterility of the hybrid females whose *pseudo-obscura* chromosomes were all derived from an old marked stock was due entirely to the genetic insufficiency of that particular set of *pseudo-obscura* chromosomes in relation to those of the *persimilis* species, it would at least be expected that some of the eggs of these females would contain either all *pseudo-obscura* or all *persimilis* chromosomes; such eggs would give rise, even when fertilized by males of the

old marked stocks, to individuals containing a genotype either wholly of *pseudo-obscura* composition, or of hybrid composition like the mother's, both of which on purely genetic grounds would be expected to be viable. About twenty-seven such backcross matings of individual hybrids containing the chromosome complement of the w^5 stock (to give but one example) failed to give any offspring at all, even at the larval stage. Moreover, the eggs laid by these hybrids invariably turned brown after 24 hr.

The above objection has less validity than would at first appear, for the following reasons:

(1) The expectation of recovering even one chromosome intact in the gametes is greatly reduced by the fact that crossing-over in the portions of the hybrid chromosomes where the gene sequence is identical is abnormally high (MacKnight, 1937); the chance that all four major chromosomes in a given pronucleus should be non-cross-overs as well as homogeneous with regard to species, must be very small indeed. It would require not only fairly large-scale experiments but also very favourable food conditions for such rare recombinants to be obtained. As is well known it is almost impossible with ordinary techniques to maintain healthy food conditions when the females are highly sterile, and slow in laying.

(2) The theoretical expectation of recovering any particular type of recombinant in a given cross is necessarily modified by several other considerations, notably the hatchability of the egg from which the individual arises, and the degree to which hatchability depends upon the genetic constitution of either or both parents. In the case of the w^5 stock it was found that eggs laid by these females, when fertilized by males of the same stock, had only an average hatchability of 23%. A new white-eyed stock, however, made up by outcrossing w^5 to wild type for four generations, gave an average of 69.7% hatchability. It is thus clear that the hatchability percentage is largely dependent on the genotype, and that the genotype of the old w^5 is not a favourable one in this respect even within the pure species. The existence of so many factors operating against the emergence of the extremely rare recombinants expected to be viable may sufficiently explain why none has been actually recovered. Whether or not there exists an additional factor of 'maternal effect' as claimed by Dobzhansky (1937) which causes the offspring of hybrid females to be less viable than those of females of pure species (genotypes being equal) would be extremely difficult to demonstrate conclusively, since it is impossible to recognize completely any genotype of the backcross generation in such a way as to be able to measure its viability apart from the supposed maternal effect. Arguments based on the viability of average genotypes can, in the nature of the case, only beg the question.

As the type and degree of hybrid sterility, however, appeared to be a sort of measure of the genetic divergence of the w^5 and other marked stocks from the wild genotype, it was thought desirable to carry the analysis further. A scheme of repeated intraspecific outcrosses of w^5 to Texas combined with a parallel scheme of interspecific crossing with *persimilis* and backcrossing of hybrid females was arranged so as to show whether the gradual introduction of Texas autosomes into the w^5 genotype would be accompanied by a proportional and systematic increase in fertility in the hybrids. If this were the case it would show conclusively that the genetic divergence between w^5 and Texas was of a very complex nature and included genes on all the chromosomes. The form in which some of these genes existed in the w^5 stock would be shown to interact less successfully with the *persimilis* genotype than did their alleles in the Texas stock. The results given in Table 1

show that this was in fact the case. The systems of inter- and intraspecific crossing used are shown in Figs. 1 and 2.

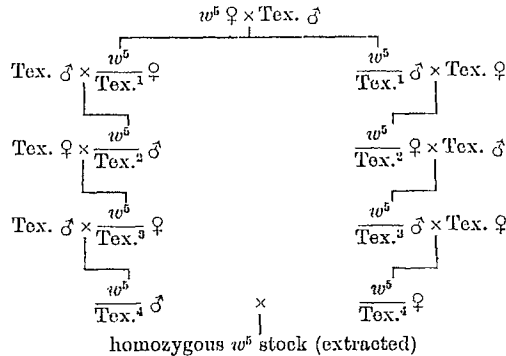


Fig. 1. Intraspecific outcrossing (*pseudo-obscura*).

[The formulae $\frac{w^5}{\text{Tex.}^1}$, $\frac{w^5}{\text{Tex.}^2}$, etc., when applied to females indicates that they are heterozygous for the w^5 marker, and that the rest of their genotype is the result of one, two or more outcrosses to Texas. The same applies to the males except that their one X-chromosome in all cases contained the w^5 marker.]

Backcross no.	Species cross no.	
	<i>D. pseudo-obscura</i> w^5 ♀ × <i>D. persimilis</i> + (Lassen) ♂	1
2	$\frac{w^5}{\text{Lass.}}$ ♀ × (a) w^5 ♂, (b) Tex. ♂, (c) $\frac{w^5}{\text{Tex.}^1}$ ♂ × Lass. ♀	3
4	$\frac{\text{Lass.}}{w^5/\text{Tex.}^1}$ ♀ × (a) w^5 ♂, (b) Tex. ♂, (c) $\frac{w^5}{\text{Tex.}^2}$ ♂ × Lass. ♀	5
6	$\frac{\text{Lass.}}{w^5/\text{Tex.}^2}$ ♀ × (a) w^5 ♂, (b) Tex. ♂, (c) $\frac{w^5}{\text{Tex.}^3}$ ♂ × Lass. ♀	7
8	$\frac{\text{Lass.}}{w^5/\text{Tex.}^3}$ ♀ × (a) w^5 ♂, (b) Tex. ♂, (c) $\frac{w^5}{\text{Tex.}^4}$ ♂	

Fig. 2. Scheme of species crosses and backcrosses.

[Cross no. 1 is the species cross: w^5 stock *pseudo-obscura* ♀ × wild-type *persimilis* (Lassen) ♂. Nos. 3, 5 and 7 are the reciprocal species crosses: *persimilis* (Lassen) ♀ × white-eyed *pseudo-obscura* ♂. These white males, however, are not stock males but were derived from the intraspecific outcrosses of w^5 stock to Texas wild type (see Fig. 1). Thus the male formulae, $\frac{w^5}{\text{Tex.}^1}$, $\frac{w^5}{\text{Tex.}^2}$, etc., indicate that the white males in question were obtained from the 1st, 2nd and 3rd, etc., outcross to Texas. Crosses no. 2, 4, 6 and 8 are backcrosses of the species-hybrid females heterozygous for the *pseudo-obscura* w^5 marker. In cross 2, the w^5 marker was obtained from a w^5 stock female (of cross no. 1) but, in crosses 4, 6 and 8, from white males of the intraspecific outcrosses to Texas. Thus $w^5/\text{Tex.}^1$, $w^5/\text{Tex.}^2$, etc., in the hybrid females' formulae indicate that the marker gene, white⁵, was introduced into the hybrid female by a white male obtained from the 1st, 2nd, 3rd, etc., outcross to Texas. The hybrid females of crosses 4, 6 and 8 therefore have a good chance of carrying one or more autosomes of the Texas wild-type stock instead of only the autosomes of the w^5 stock, as is the case of the hybrid female of cross no. 2. Each type of hybrid female was backcrossed to three different types of *pseudo-obscura* male, (a), (b) and (c), as shown in the figure and in Table 1. The type of males used in crosses 2b, 4b and 6b were also used to make the species crosses 3, 5 and 7, but the same individual males were not, of course, used for both matings.]

Matings 1, 3, 5 and 7 of Fig. 2 were species crosses. Matings 2, 4, 6 and 8 were backcrosses of hybrid females heterozygous for w^5 to (a) males of the old w^5 stock, (b) to Texas wild-type males, and (c) to w^5 males extracted after one or more generations of outcrossing to Texas wild type (represented by the formulae $\frac{w^5}{\text{Tex.}^1}$, $\frac{w^5}{\text{Tex.}^2}$ and $\frac{w^5}{\text{Tex.}^4}$ respectively). The results of matings of series 6 are not shown, as the records were incomplete.

Table 1 shows the high correlation between the degree of wild-type *pseudo-obscura* chromatin present in the cross (substituted for that of the w^5 stock by outcrossing, as shown in Fig. 1) and the degree of fertility of the hybrid female.

In the first three matings shown in Table 1 (*2a*, *4a* and *8a*), the hybrid females were all backcrossed to w^5 males of the old stock. The hybrid females themselves were all heterozygous for the w^5 marker, but whereas the first set (*2a*) were hybrids from the straight cross (w^5 stock ♀ × *persimilis* ♂ Lassen), those of *4a* were the offspring of a white male obtained by outcrossing a w^5 female to a wild-type Texas male, and those of *8a* had had as father a white male obtained after three generations of outcrossing.

The twenty-seven females of *2a* gave no offspring at all. Only three out of seventeen of *4a* gave offspring, but these were very few and included only wild-type females, all of which showed the typical deformities associated with hybrid backcrosses (Lamy, 1943). Two out of fourteen of the *8a* series were fertile, giving an average of seven offspring per pair, including a few males, and some not-deformed individuals.

Table 1. Results of hybrid backcrosses

Mating no.	No. pair fertile mated	Parents		Offspring				Av. off. per pair	No. ♂♂ per 100 ♀♀	Prop. $w : +♂$	% deformed progeny
		♀	♂	+♀	w ♀	+♂	w ♂				
<i>2a</i>	0/27	Lass. w^5	× w^5	—	—	—	—	—	—	—	—
<i>4a</i>	3/17	Lass. $w^5/\text{Tex.}^1$	× w^5	4	—	—	—	1.3	—	—	100
<i>8a</i>	2/14	Lass. $w^5/\text{Tex.}^3$	× w^5	11	—	3	—	7	27	—	93
<i>2b</i>	5/5	Lass. w^5	× Tex.	66	—	32	1	19.8	50	1 : 32	38
<i>4b</i>	10/10	Lass. $w^5/\text{Tex.}^1$	× Tex.	387	—	172	28	58.7	52	1 : 6	24
<i>8b</i>	6/6	Lass. $w^5/\text{Tex.}^3$	× Tex.	271	—	122	17	68.3	51	1 : 7	8
<i>2c</i>	7/10	Lass. w^5	× $\frac{w^5}{\text{Tex.}^1}$	14	—	28	1	6.1	207	1 : 28	57
<i>4c</i>	9/9	Lass. $w^5/\text{Tex.}^1$	× $\frac{w^5}{\text{Tex.}^2}$	115	20	109	17	28.8	94	1 : 6	26
<i>8c</i>	9/10	Lass. $w^5/\text{Tex.}^3$	× $\frac{w^5}{\text{Tex.}^4}$	244	22	242	38	60.6	105	1 : 6	12

Thus, though the backcross to the stock w^5 male is always difficult, the presence of some wild-type *pseudo-obscura* chromatin in the hybrid female increases the likelihood of obtaining offspring in this mating.

The effect of the genotype of the hybrid female on the result of the cross is also seen in the second series of matings where the males used throughout are Texas, and the hybrids again differ in the amount of wild-type *pseudo-obscura* chromatin they contain. Where there is none at all (*2b*) the fertility is lowest, the number of deformed offspring greatest and the survival of white males very rare. With the first degree of outcrossing in the *pseudo-obscura* parent of the hybrid, the fertility of the hybrid is increased three times, the number of abnormal progeny falls considerably, and the expectation of white sons is five times greater. The same trend is continued in the next mating where the white-eyed father of the hybrid had been obtained after three generations of outcrossing.

The third series (*2c*, *4c*, *8c*) shows the result of introducing various amounts of wild-type *pseudo-obscura* chromatin in both the hybrid female and the male used in the back-

cross. In 2 (*c*) half the autosomes of the white male are derived from the Texas wild stock, but all the *pseudo-obscura* chromatin present in the hybrid female is derived from the w^5 stock. There are several interesting points about this mating. The amount of wild-type *pseudo-obscura* chromatin present in the cross is less than that of 8*a* but probably more than that of 4*a*. The average fertility of the hybrids was five times greater than 4*a*, being nearly equal to that of 8*a*, and a much larger proportion of the females gave offspring, compared with either of the *a* matings. Moreover, a white-eyed male appeared in this cross, and the number of deformed individuals among the offspring was very much less than in the *a* matings. It is clear that part of the success of this cross must be attributed, not directly to the amount of wild-type chromatin available to the offspring, but to the effect of this material on the fertility or vigour of the white male which carried it. The same effect in a greater degree is seen in the mating 2*b*, where the male used in the backcross is pure Texas and the hybrid female is one obtained from the straight cross ($w^5 \times persimilis$); this cross represents the weakest (or most sterile) hybrid genotype and the strongest *pseudo-obscura* male genotype, and shows the full effect of the latter on raising the fertility of the former. Both these matings, 2*c* and 2*b*, show the full contribution of the male genotype to the success of the cross by way of its physiological effect on the male, but when these are compared with the other crosses in which the hybrid female also contained some degree of wild *pseudo-obscura* chromatin, it becomes apparent that the genotype of the female also has a very marked effect on the number and quality of the offspring she produces.

Where the amount of wild-type chromatin is increased progressively in both males and females there is, according to expectation, a sharp rise in total offspring and an equally sharp decline in the proportion of abnormal progeny, in proportion to the total amount of Texas chromatin available to the offspring.

The survival of white offspring is always much below expectation, but in view of the lack of control of the large *X*-chromosome, the influence of the probable residue of the w^5 *X*-chromosome cannot be assessed.

What emerges clearly from these results is that the fertility of the hybrid females is in direct relation to the genetic constitution of both the hybrid females themselves and of the males to which they are backcrossed. This is also broadly true of all breeding experiments within species (compare, for instance, Reiff, 1945), though it is necessarily complicated and exaggerated in species crosses.

CONCLUSION

The results described above may be considered under two separate headings: (1) the degree to which hybrid fertility is affected by the particular genotypes of the parents, and (2) the fact that within species the genotypes of certain strains apparently undergo more rapid divergence compared with other strains. It is of interest, in view of Dobzhansky's report (1937), that the divergence in the particular strain analysed in this paper (w^5), which resulted in a considerable reduction in the average fertility of the flies, had a similar and very marked effect on the fertility of the hybrid females. In other words, a given form of genetic divergence was expressed both intraspecifically and interspecifically in the same way (reduced fertility), strongly suggesting that the complex of fertility genes in both species must be roughly equivalent. This would, in fact, be expected where the hybrid female is usually of a good fertility, as in this species cross. Nevertheless, it must be

pointed out that what we are dealing with is not necessarily a system of homologous genes concerned with fertility in the two species. If this were so the cause of the complete failure of the backcross to the divergent strain would still be very unclear; in view of the above results, however, it may be taken to suggest that there are important differences between the two species in the sphere of genic interaction which can be easily upset by divergent shifts within one of the species. This aspect of the subject has already been discussed in a former paper. The particular question of the cause of rapid divergence within species will be dealt with in a future paper.

SUMMARY

The sterility of certain *Drosophila pseudo-obscura/persimilis* hybrid females is shown to be directly correlated with the genotype as a whole of the *pseudo-obscura* parent. Progressive alteration of the genotype by outcrossing resulted in increased fertility of the hybrids.

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