

FERTILITY AND STERILITY IN DROSOPHILA AMPELOPHILA

III. EFFECTS OF CROSSING ON FERTILITY IN DROSOPHILA¹

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ONE DIAGRAM

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¹ This part of the work was carried out at the Marine Biological Laboratory at Woods Hole, Mass. The writer is indebted not only to the management of the Station, but also to the Zoological Department of Columbia University for placing at his disposal every opportunity for carrying forward the present study. I wish to express my appreciation to Prof. T. H. Morgan for the unfailing interest he has taken in these studies.

INTRODUCTION

This paper, which is the third of the series on "Fertility and Sterility in *Drosophila ampelophila*," considers the evidence that bears on the effects of crossing on fertility when: (1) Stocks from the same source (descended from the same grandparents) separately inbred are crossed; (2) Stocks from different sources separately inbred are crossed; (3) When the mutations, having pink eyes and white eyes are crossed with each other and with a wild stock which has been inbred (brothers and sisters) for 26 generations.

The evidence so far presented goes to show that some of these stocks have, up to the time the present experiments were carried out, lost in fertility on inbreeding. The evidence, however, does not show conclusively that the loss is due to the process of inbreeding as such. The fact remains, that the different strains did up to this time lose in fertility on continuous inbreeding, and it will now be shown what effect out-crossing has on these reduced strains. It is to be recalled that 'fertility' is here used in the sense of eggs that reach maturity.

There are some experimental data that bear out the belief that the crossing of different races or strains is beneficial to the *fertility of the stock itself*. This relation has been demonstrated beyond any doubt in the second part of this paper in so far as it applies to the truncate mutant for the fertility of the female was more than doubled when mated to a wild stock, while the fertilizing powers of the truncate male were more than three times as great with a wild female as compared to his own mate.

I wish now to consider the evidence that bears on the question as to whether or not a rise in fertility will result when one strain of *Drosophila* is crossed with any other strain. The evidence deals especially with the fertility of two mutations and two wild stocks that originally came from two different sources. It will facilitate treatment of this subject to give a brief résumé of the history of these stocks.

HISTORY OF THE STOCKS USED IN CROSSING

1. *The Woods Hole stock*

This is a wild stock that originally came from Woods Hole, Massachusetts. How long it had been in captivity I do not know, but when I took charge of the stock it showed a fertility of 77.3 per cent. The stock was tested at various times throughout the year 1912-1913. The behavior of the fertility of this stock is given in table 1. The stock was cultured *en masse*.

TABLE 1

Showing the fertility of the Woods Hole stock

TIME TESTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	PER CENT FERTILITY
September 11 to September —, 1912.....	1402	1084	77.3
September 16 to October —, 1912.....	1622	1227	75.0
January 20 to February 14, 1913.....	2085	1421	70.0
June 28 to July 15, 1913.....	917	581	63.4
July 15 to July 28, 1913.....	531	317	59.7
August 7 to August 24, 1913.....	601	431	71.7
August 17 to September 4, 1913.....	856	715	83.6
October 21 to November 11, 1913.....	467	341	73.0

2. *The inbred stock*

This stock originally came from Falmouth, Massachusetts. During the course of the experiments brothers and sisters have been selected and paired for over 26 generations. Its productivity was at the beginning of the experiments relatively high as shown by the large number of offspring produced. On inbreeding the stock gradually dropped in productivity as shown in table 2.

TABLE 2

Showing the productivity of the inbred stock in successive generations of close inbreeding

GENERATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. offspring produced....		368	209	190	184	65	119							159

This evidence taken by itself does not prove conclusively that the loss in productivity was due to a decrease in the fertilizing power of the gametes. That this is a very probable interpretation however is shown by the fact that in the F_{14} generation when the productivity dropped to 159 per pair their fertility was only 32 per cent. And yet the flies of the F_{14} generation were producing twice as many fertile sperm and twice as many fertile eggs as shown by outcrossing into the truncate stock (Part II, diagram E).

3. I_2 and I_3 stocks

In the fifth generation of the inbred stock, when they were producing 184 offspring to the fertile pair, I set aside two different bottles besides a number of pairs that were kept to continue the inbred stock. All of these flies had descended from the same grandparents, a single pair of the third generation. In one bottle there were three females and four males. This I shall designate as I_2 . In the other bottle there were ten males and twenty-two females. This I shall refer to as I_3 . These flies were set apart April 2, 1912, and received no attention except that the flies were transferred to clean bottles about every three weeks and fresh food added from time to time. It is important to bear in mind that we have three stocks descended from the same germ-plasm.

4. *The white-eyed stock*

This stock arose as a mutation from the inbred stock early in the history of the strain. This stock received the same treatment as I_2 and I_3 .

5. *The pink-eyed stock*

This is an eye mutation that arose in one of Morgan's cultures.² It had been bred for some three years. I received my stock from Mr. Liff, a graduate student in the department, who has made a study of their productivity. This stock was bred in mass culture for about four months when the present experiments were carried out.

² Science, vol. 33.

We have then six different strains, two from different localities and some that had the same germ plasm. I now propose to examine the fertility of the different stocks and their behavior on crossing. The combinations were made up as shown in diagram A.

METHODS

In order to get an exact measure of fertility the eggs were isolated as described in Part II. From the stock bottle in each case 15 virgin females and 30 young males were selected. The flies that hatched from June 19 to June 26 were separated every twelve hours from the stock bottles. There can be no question as to the virginity of the females. The males and females were kept in separate bottles and the different combinations made up on June 26. An epidemic of mating took place in all the bottles a short time after the flies came out from under the influence of the ether.³ I commenced to isolate the eggs two days later. This process was carried out in the same way as in previous experiments with one exception. The weather was very warm and the larvae emerged from the egg in less than twenty-four hours. In order to exclude this source of error I added another bit of food after isolating the eggs. This served as food for the parents. After six or eight hours this food was removed and a new bit added from which the eggs were later isolated. Tables 3a, 3b, and 3c give the number of eggs isolated each day and the corresponding number that hatched.

DISCUSSION

A study of diagram A brings out the essential relations that concern the questions propounded at the beginning of this paper. 1. What is the effect on fertility when germ plasm originally from the same source separately inbred for several generations is recombined by crossing? It will be recalled that I, I₂ and I₃ represent in this case the stocks under study. It is to be noted that the three stocks although originally brothers and sisters and descended from

³ Mr. A. H. Sturtevant has shown that the mating habit in this species is largely associated with the sense of smell and this fact probably accounts for the phenomenon here observed.

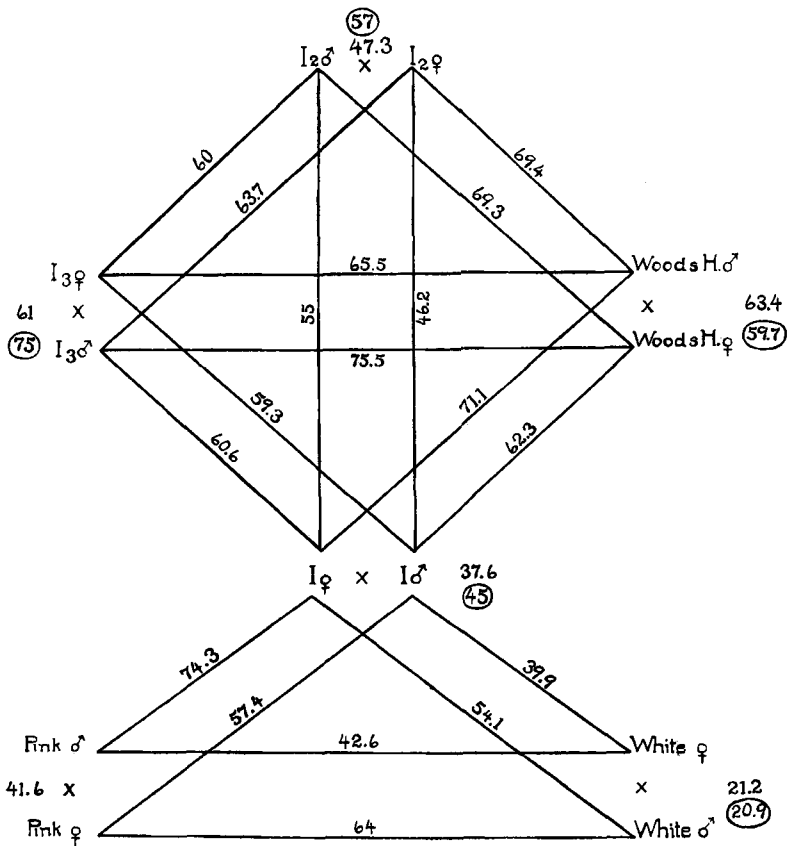


Diagram A Based on the foregoing data, showing different combinations and the fertility in each case.

the same grandparents, show different degrees of fertility. The inbred stock in which brothers and sisters had been continually selected had lost most in fertility. In the crosses it will be observed that there is no sudden rise in fertility as one might expect but, on the other hand, a peculiar relation exists in that the stock in each case having the highest fertility is able on crossing to bring the fertility of the lower stock up to its level and this is true whether through the male or through the female.

TABLE 3 a

Showing the number of eggs isolated day by day and the corresponding number that hatched from each of the combinations. The first letter in each combination represents the stock from which the female was taken, the second letter the stock from which the male was taken. *T* = truncate; *I* = inbred, *I*₂ = second inbred stock from 3 males, 4 females; *I*₃ = third inbred stock from 10 males, 22 females. *WH* = Woods Hole stock; *P* = pink-eye stock; *W* = white-eye stock

DATE	¹ (control) <i>T</i> × <i>T</i>		² <i>I</i> ₂ × <i>I</i> ₂		³ <i>I</i> ₃ × <i>I</i>		⁴ (control) <i>I</i> ₂ × <i>I</i> ₂		⁵ <i>I</i> × <i>I</i> ₂		⁶ (control) <i>I</i> ₂ × <i>I</i> ₂		⁷ <i>I</i> ₂ × <i>WH</i>	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
June 28.....	0	0	48	27	37	21	80	39	60	25	40	23	25	21
29.....	36	7	21	17	34	24	35	24	47	27	61	29	56	42
30.....	56	14	50	32	29	20	55	32	60	31	36	16	0	0
July 1.....	50	13	30	19	40	26	40	29	48	25	60	26	38	31
2.....	37	4	25	12	40	17	25	7	32	21	71	18	20	6
3.....	48	2	40	18	33	20	33	14	0	0	49	25	22	9
4.....	45	7	30	14	65	26	22	11	60	26	50	23	26	13
5.....	52	6	56	27	57	39	73	41	52	21	85	20	50	33
6.....	42	10	64	36	36	16	50	14	26	7	75	31	50	34
7.....	50	11	75	51	50	29	67	55	75	61	55	35	55?	41
8.....	20	9	33	23	28	24	17	15	36	20	24	10	12	10
9.....	22	5	28	21	24	12	30	25	42	26	44	17	0	0
10.....	43	4	75	30	60	26	35	24	50	26	75	40	25	12
11.....	60	13	40	24	0	0	50	35	60	25	60	37	60	46
12.....	30	8	50	39	75	60	21	19	75	44	75	38	37	24
13.....	9	5	25	16	40	22	20	16	55	34	65	41	13	6
14.....	0	0	30	26	33	25	0	0	35	30	40	26	17	4
15.....	0	0	20	11	20	9	0	0	15	8	20	11	16	7
Total.....	600	118	740	443	701	416	653	400	828	457	985	466	522	339
Per cent.....	20		60		59.3		61		55		47.3		61.5	

TABLE 3 b

⁸ (control) WH × WH		⁹ I ₂ × WH		¹⁰ I × WH		¹¹ I × I ₂		¹² I ₂ × I ₂		¹³ (control) I × I		¹⁴ I ₂ × I		¹⁵ WH × I ₂		¹⁶ WH × I	
Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
36	20	47	30	35	25	22	11	25	11	19	4	38	20	53	46	35	21
18	11	31	20	60	45	24	20	21	11	18	13	42	21	42	30	65	33
40	24	27	16	50	26	45	25	38	28	71	37	25	12	62	57	62	51
28	19	35	22	45	23	42	22	0	0	26	8	60	33	48	38	40	36
18	7	20	7	25	13	30	16	35	25	30	13	40	24	50	27	19	13
15	10	61	42	45	29	28	13	41	19	17	6	29	13	100	50	31	20
55	28	8	4	25	19	35	17	50	26	31	7	45	15	50	30	65	23
51	28	43	25	63	53	68	27	90	37	60	15	64	27	100	63	35	15
80	47	50	29	46	28	30	18	67	50	40	10	75	31	52	50	40	13
80	60	41	33	75	65	80	57	66	45	40	18	50	31	100	80	90	67
100	69	23	20	32	21	30	21	12	9	45	18	38	21	64	56	45	38
60	46	35	32	51	38	30	16	31	22	22	6	23	8	57	47	40	33
76	38	0	0	64	34	50	26	35	20	50	17	65	25	72	45	75	32
60	30	60	44	60	44	40	22	40	25	50	14	60	20	60	30	60	20
55	35	70	61	100	84	80	64	75	63	60	25	50	35	68	64	70	49
63	53	40	29	40	26	20	11	60	50	43	17	39	24	75	70	55	40
42	32	45	33	40	35	35	26	34	21	15	11	30	12	40	39	50	44
40	24	30	15	40	29	40	30	20	9	20	7	30	0	40	33	30	17
917	581	666	462	896	637	729	442	740	471	657	246	805	372	1133	855	907	565
63.4		69.4		71.1		60.6		63.7		37.6		46.2		75.5		62.3	

TABLE 3 c

17 $WH \times L_2$		18 $W \times I$		19 $I \times P$		20 $P \times I$		21 (control) $P \times P$		22 $W \times P$		23 (control) $W \times W$		24 $I \times W$		25 $P \times W$	
Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
45	30	41	22	0	0	10	6	36	22	50	38	28	11	10	5	60	46
53	44	40	18	0	0	0	0	30	9	16	4	20	4	14	10	38	26
60	48	18	9	32?	11	10	5	35	23	15	5	33	8	31	23	80	63
50	33	36	14	20	14	21	17	32	17	26	16	25	10	28	13	61	38
35	21	22	8	14	10	26	16	34	7	24	4	7	4	29	17	42	15
49?	26	31	11	7	5	20	10	56	15	7	2	42	8	22	13	47	24
82	42	55	15	10	3	55	28	20	4	43	12	25	1	17	2	30	7
58	38	20	7	47	31	80	53	75	28	14	2	40	3	85	50	85	38
48	31	35	6	19	16	18	6	50	15	17	8	10	0	21	9	41	19
90	76	27	9	80	60	40	23	40	19	40	24	50	9	23	11	60	47
75	66	8	0	37	34	0	0	7	5	10	5	40	7	34	16	31	21
54	41	15	3	26	19	17	13	24	17	13	7	50	13	27	11	50	43
65	30	21	7	60	44	43	20	46	10	35	8	29	2	60	31	81	50
50	10	60	20	60	46	70	31	60	17	50	21	65	13	70	30	70	35
65	59	50	32	70	60	85	54	80	44	45	23	55	19	60	41	40	40
50	41	43	25	30	24	60	29	20	12	22	10	65	12	45	17	50	39
30	28	33	21	40	36	40	30	50	24	42	18	35	7	27	19	26	17
20	14	35	7	20	12	20	12	40	18	40	10	30	7	10	4	18	15
979	678	590	234	572	425	615	353	735	306	509	217	649	138	613	332	910	583
69.3		39.7		74.3		57.4		41.6		42.6		21.2		54.1		64	

The second question under consideration is; What is the effect on fertility when stocks from different sources separately inbred are crossed? The relations expressed by the right hand side of diagram A throw some light upon this question. In two of the combinations high fertility brought the low fertility up to its level while in four of the combinations there is an appreciable rise in fertility beyond that shown by the parents, although by no means as high as one might expect from the history of the crosses made with the truncates in Part II. But since the rise in fertility occurs here in the combinations expressed by the right side of the diagram and not those on the left it looks as if the rise in fertility in this case is significant. It must be admitted, however, that the rise in fertility in these crosses is not great enough to base a final conclusion upon, in regard to a point as far-reaching as this. It is to be remembered that Woods Hole, Massachusetts, is only four miles distant from Falmouth and it is altogether probable that the two strains had not been separated by many generations when taken into captivity. Consequently, this material would present after all a picture very much like the first case. If the environment can influence the different strains in respect to the 'factors' that bring about fertility the influence in this case has been slight, and, after all, from stocks so closely related this is probably what we should expect to find. In any case it is certain that the high-producing stocks can bring the fertility of the low-producing stocks to their level whether descended from the same or from different germ plasms. It might seem from this (although I am far from contending at present that such is necessarily the case) that there is a set of factors of some sort for fertility and that when a loss in fertility occurs in a stock (other than that which occurs in the case of mutations) it is the same set of factors that is lost or is changed and this accounts for the fact that there is no rise in fertility on crossing. The stock that has the highest fertility (the largest number of factors) acts as a dominant character and brings the lower set of factors up to its level. In the case of a sudden rise in fertility on crossing such as occurs in the case of some of the mutations it is probable that the stocks have lost different factors for

fertility, and that crossing gives the proper constellation of factors for a marked rise in fertility.

It must be frankly admitted that this explanation at best is only tentative and that the door must be left open for further investigation. The possibility of transmissible lethal factors is not to be overlooked.

The third question under consideration relates to the effect on fertility of crossing certain mutations, namely, pink-eye and white-eye and the effect on fertility when these are crossed into the inbred stock. The triangular portion of diagram A shows that there is a rise in fertility in all cases. In four of the six cases the rise in fertility is very high. This is analogous to what happens in the case of the truncates when crossed into other stocks.

I wish here to add the data from an experiment that has some bearing on the foregoing considerations and also some bearing on the question of inbreeding. The object of this experiment is to serve as a check upon the controls used in the foregoing experiments, namely, Woods Hole, white-eye, I_2 , I_3 and the inbred stocks. This experiment was carried out in the same manner as the foregoing. The egg counts began the same day that the former experiment closed, July 15, 1913. The number of eggs isolated, together with the corresponding number that hatched, is given in table 4.

I have placed the percentage of fertility of these stocks in diagram A and enclosed the numbers in circles. It will be noted that the Woods Hole and white-eyed stocks remain practically the same as in the previous experiment. There is a marked rise in fertility, however, in case of the three stocks originally from the same germ plasm. Later (Aug. 7 to 24, table 1) the Woods Hole stock was tested. Its fertility had risen from 59.7 to 71.7. The Woods Hole stock was again tested August 17 to September 4. This stock now gave a fertility of 83.6. The fact is that all my stocks at this time showed a marked rise in fertility, as is evidenced by the experiments to be dealt with in the next paper. Even the truncate stock which had been tested many times through the year and had varied from 20 to 26 per cent now gave a fertility

of 31 per cent. Table 1 shows that in the case of the Woods Hole stock the fertility gradually fell throughout the year. I fully expected to find that the fertility would be continually reduced on inbreeding, and yet the fertility of the stock rose in August and September until it was actually higher than it was during the September of the previous year by 6.3 per cent. The meaning of this is obscure. It will be recalled that Castle found seasonal fluctuations in productivity in his flies. The period, however, in which low productiveness prevailed in his stocks corresponds in a general way with the period of high fertility in my stocks.

In the light of this evidence it would seem that inbreeding as such cannot be the *vera causa* of the low fertility that usually accompanies the process. It would seem from this evidence and the evidence presented by Moenkhaus that the fertility of a stock could be maintained, and the closest inbreeding practiced, provided

TABLE 4

Showing the fertility of the Woods Hole, white-eyed, inbred, I₁ and I₂ stocks. 1A = Woods Hole stock; 2A = white-eyed stock; 3A = I₁ stock; 4A = I₂ stock; 5A = inbred stock, F₂₁ generation

DATE 1913	1A		2A		3A		4A		5A	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
July 15	95	65	75	21	70	58	53	33	75	32
16	50	34	33	4	40	31	35	21	67	31
17	45	30	50	15	40	35	50	40	30	14
18	44	31	30	8	50	42	60	41	32	15
19	29	14	51	20	45	34	50	26	50	26
20	30	10	50	11	66	40	45	22	30	11
21	31	15	31	2	40	33	25	7	35	16
22	41	24	25	3	35	30	35	14	12	6
23	21	11	31	4	25	19	40	22	20	6
24	30	21	26	3	17	13	40	26	16	9
25	25	12	25	3	17	6	22	8	22	12
26	50	29	25	5	32	27	22	16	33	18
27	25	13	11	2	37	26	25	14	20	10
28	15	8	35	5	30	18	25	12	25	6
Total.....	531	317	498	106	544	412	527	302	467	212
Per cent.....	59.7		20.9		75.7		57.3		45.4	

the proper combinations were made. It seems probable in the case of fertility, as in many other characters, that inbreeding gives a chance for defects to be brought to the surface; and that low fertility is likely to accompany close inbreeding provided it is not guarded by rigorous selection. When a stock has reached a low degree of fertility it seems strange that that same stock should be able to rise again in fertility. Yet this is exactly what may happen. Take for example the truncate fly which has been selected for 75 generations and has its fertility reduced to about 20 per cent; and yet that fly can throw a form, the long wings, the fertility of which is more than twice as great as its truncate brothers and sisters.

The fact that different individuals, brothers and sisters of the same stock, should differ in such a marked degree (so that one is actually able to separate the more fertile ones from the less fertile flies by inspection) is submitted as evidence to show how selection may operate in controlling the fertility in these strains.

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