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FERTILITY, MATING BEHAVIOUR AND SEXUAL SELECTION IN *DROSOPHILA SUBOBSCURA*

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1. INTRODUCTION

Hollingsworth & Maynard Smith (1955) studied an inbred line of *Drosophila subobscura*, referred to as the O line, in which the males were infertile. When females known to be fully fertile were mated to O males, a large proportion of the eggs laid failed to hatch; the fraction of eggs from such a mating which hatched was taken as a measure of the fertility of the male parent. Further study of this male infertility is made more difficult by the great variation in the fertilities of different O males, which may range from 1 to 85%. Such variation could be due to:

- (i) Genetic differences between males,
- (ii) Differences in the environmental conditions in which the males were raised, or

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(iii) The age at which the male was mated, the number of occasions on which the male had mated previously, and the period elapsing between mating and the collection of eggs.

It was decided to study first the effects of the third group of factors, since these are the easiest to control. This paper describes such an investigation; it still leaves much of the variation in fertility unexplained, but it has shown that **O** males are deficient both in the quantity and in the quality of the sperm produced when compared to outbred males.

It was necessary to watch the mating of the pairs whose fertility was measured. As a result of observing both the fertility and the mating behaviour of individual flies, some surprising facts have been discovered concerning the conditions influencing mating behaviour in this species.

A case of sexual selection in *D. subobscura* has previously been demonstrated by Rendel (1945). Another case, which may have a closer resemblance to the selection occurring in wild populations, is described here, and it is suggested that one feature of the courtship behaviour of females may have evolved because it results in a female mating with one conspecific individual rather than another, the 'selection' of a mate being such that the female leaves a greater number of progeny.

2. METHODS AND TERMINOLOGY

All the females used were F_1 hybrids between two inbred lines, **B** and **K**, and are referred to as **B/K** hybrids.

Four types of males have been used:

- (i) Outbred males, i.e. **B/K** F_1 hybrid males.
- (ii) Inbred males, taken from the F_{18} of the **O** brother-sister mated line.
- (iii) Inbred testis-less males, which are the sons of females homozygous for the recessive mutant *grandchildless* (Spurway, 1948). These males have no testes, but the external genitalia, accessory glands and ducts are normal; they are highly inbred, since the stock can be kept only by crossing close relatives, and carry the sex-linked mutant *vermilion* (bright red eyes), which is present in the *grandchildless* stock.
- (iv) Outbred testis-less males, obtained by crossing females from the *grandchildless* stock to **B/K** males. Like the previous type, they have *vermilion* eyes and no testes, but they are not inbred.

The testis-less males were used only in the experiments on behaviour.

A number of **O** and **B/K** males, 3 days after eclosion, were paired with 7-day virgin **B/K** females. Where mating was observed to take place, the pair was immediately separated, and the male paired with a second virgin female. A second mating often occurred within approximately 1 hr. of the first. In this way, ten pairs of females were collected, the two members of a pair having been observed to mate with the same **O** male in rapid succession. The ten **O** males are referred to as ♂ **O**1, ♂ **O**8, etc., the first female mated to ♂ **O**1 as ♀ **O**1a, and the second as ♀ **O**1b. Similarly, six pairs of females were obtained, the two members of a pair having been observed to mate with the same **B/K** male. Such males are referred to as ♂ **B/K**1, etc., and the females as ♀ **B/K**1a and ♀ **B/K**1b, as before.

The thirty-two females were subsequently transferred every day into a fresh vial, until they died, were killed for dissection, or were remated. Each vial contained a slip of balsa wood on to which had been poured a drop of food consisting of agar and molasses, with

living yeast suspension added. The females laid eggs on this food, and, 48 hr. after the transfer of the female from a vial, the wood was removed and the numbers of eggs laid and hatched were recorded. Where only one or two eggs had hatched, the hatch was always confirmed by the presence of living larvae. All vials were kept covered with a damp cloth, under which the temperature varied from 64 to 67° F.

The ten O males were kept in separate vials, and were paired at weekly intervals with 7-day virgin females. The females mated, for example, to ♂ O1 are referred to as ♀ O1c, O1d, O1e, according to whether mating took place 1, 2 or 3 weeks after the original matings. Samples of eggs were collected from these females before discarding them.

It is therefore possible to compare the percentage hatch of eggs laid by different females mated to the same male at different times, and by the same female at different periods after mating.

3. FERTILITY

(a) *Representative females*

Any grouping of the data on numbers of eggs laid and hatched tends to obscure what is happening. Therefore the results for the first 40 days after mating for three representative females are given in Fig. 1. Considering first the females fertilized by B/K males, four periods can be recognized:

Period A, during which all or almost all the eggs hatch.

Period B, during which there is a rapid decline in the proportion of eggs hatching. This period is taken as starting on the first of two consecutive days on which the percentage egg hatch is less than 90%.

Period C, during which only an occasional egg hatches, taken as starting on the first of two consecutive days on which less than 20% of the eggs hatch, and ending on the day when the last hatched egg was recorded. This period was short or absent in some females (e.g. ♀ B/K16a, Fig. 1) and prolonged in others (e.g. ♀ O1a, Fig. 1).

Period D, during which no eggs hatch.

The boundaries between these periods have been chosen arbitrarily. It is almost certain that the reason for the low hatch during periods C and D is that only a small quantity of sperm remains in the female receptacle. This has been confirmed for some females by dissection. The failure to hatch cannot be due to the quality of the eggs produced, since on the few occasions on which such females have remated and have laid further eggs, these eggs have hatched. Females in periods C and D will be referred to as 'exhausted' females.

Females fertilized by O males differ in that an appreciable proportion of the eggs laid immediately after mating fail to hatch. This is thought to be because a proportion of the sperm produced by O males are in some way inadequate. However, there is an initial period A during which the percentage hatch for a given female is fairly constant. In such cases period B is taken as starting on the first of two successive days, on each of which the percentage hatch was lower than the mean for the whole preceding period, the difference for the two days combined being significant ($P < 0.01$).

(b) *Inadequately inseminated females*

In all, eight females (five of the original thirty-two females *a* or *b*, and three of the females *c* to *e*) proved to have been inadequately inseminated, although copulation was observed. The histories of the five original females are shown in Fig. 2. In all cases they

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laid few eggs, and of these few or none hatched. Failure to inseminate may occur because the male has exhausted his sperm in a previous mating (e.g. ♀ **B/K** 17*b*), or possibly because the male failed to produce sufficient sperm (♀ **O** 20*a* and ♀ **O** 20*b*). In four of the eight

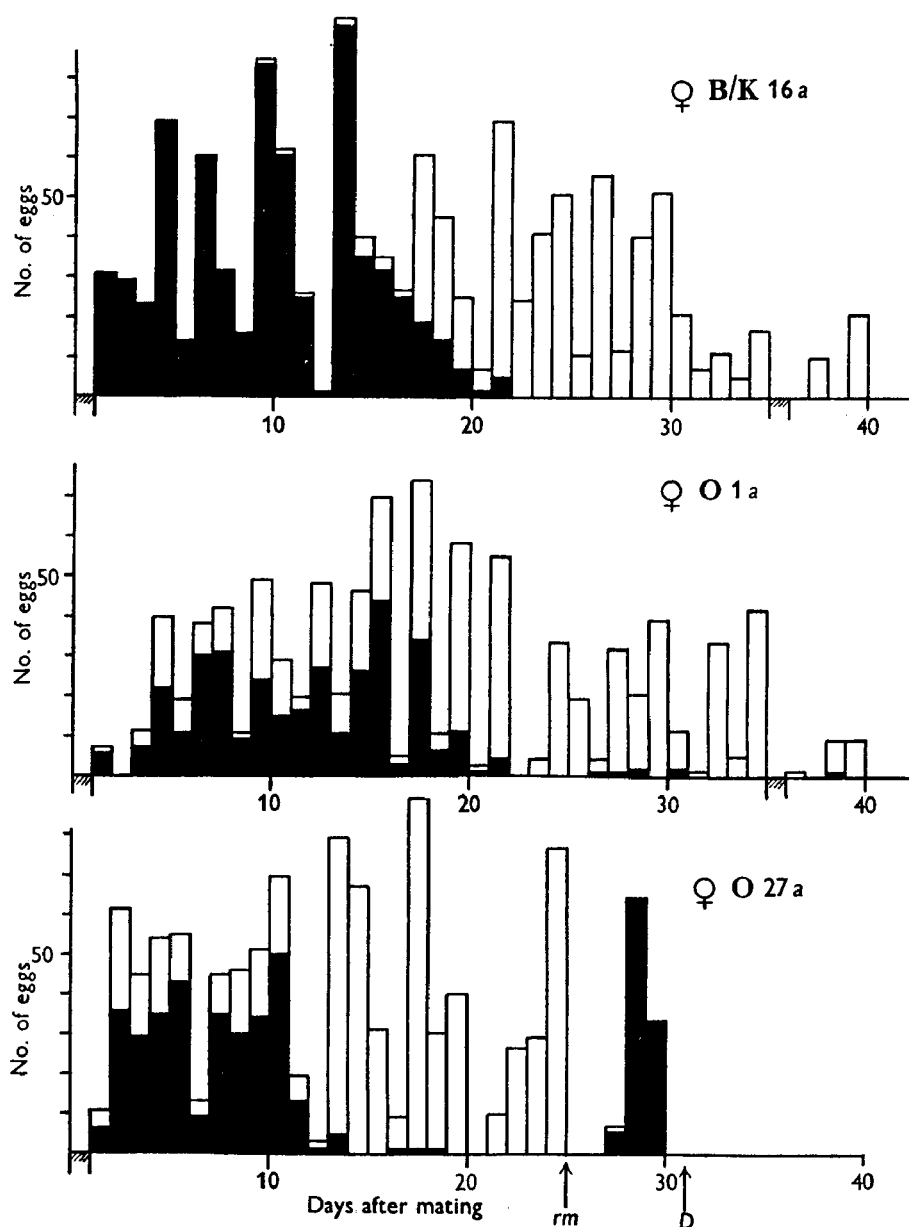


Fig. 1. Number of eggs laid daily, hatched (black) and unhatched (white), by three representative females. *D*, died; *rm*, remated to B/K male. Shading below the abscissa indicates that the eggs were not recorded during that period.

cases, the females were paired again with B/K males after a week, mating took place, and the females subsequently laid fertile eggs. In three cases the females were dissected, and proved to have few or no sperm in their receptacles. Lastly, ♀ **O** 28*b* remated after a week

with the same O male, and the mating was again ineffective. After another week, this female was paired with a B/K male, and after a number of trials mated for a third time, and laid fertile eggs.

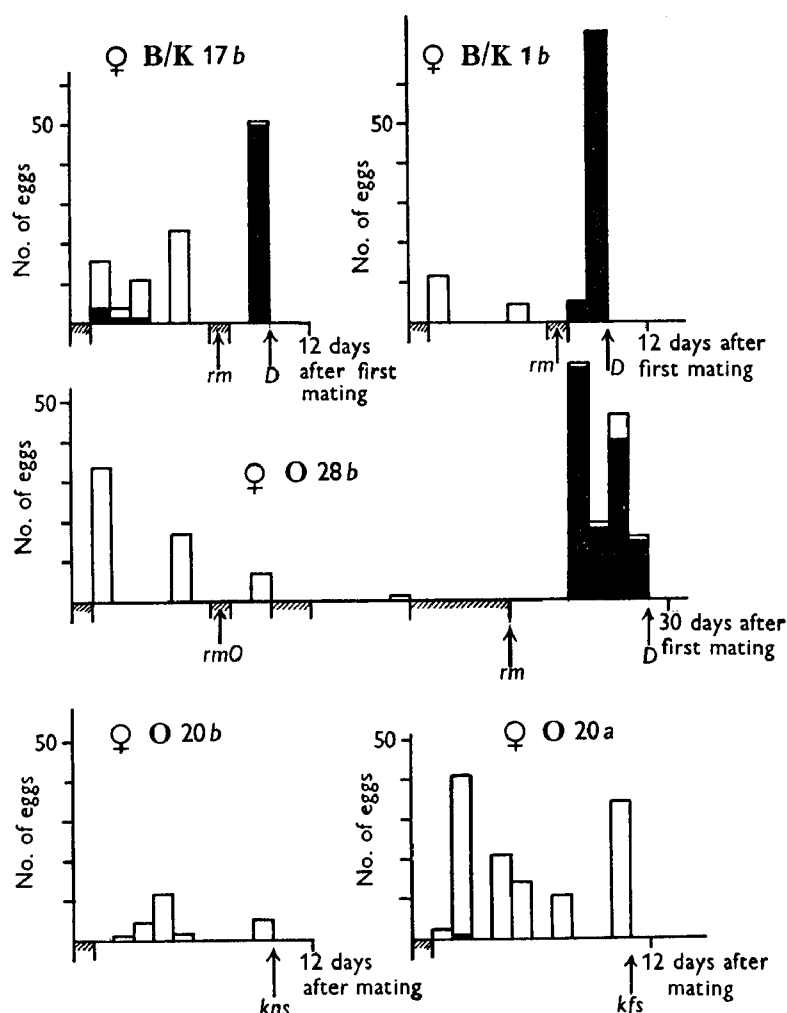


Fig. 2. Number of eggs laid daily, hatched (black) and unhatched (white), by five inadequately inseminated females. *D*, discarded; *kfs*, killed, few motile sperm present; *kns*, killed, no motile sperm present; *rm*, remated to B/K male; *rmO*, remated to O male. Shading below the abscissa indicates that the eggs were not recorded during that period.

(c) Summarized data

The results for all thirty-two females are summarized in Table 1.

(i) Adult longevity of B/K females

The data on this are unsatisfactory when compared to the results of Clarke & Maynard Smith (1955), since fewer females were used, and some were discarded in the course of the experiment. However, the females in the present experiment lived longer; eight of the original thirty-two females were alive 100 days after eclosion, and one lived for 133 days,

Table 1. Fertility of B/K females mated to B/K males and to O males

Female	Periods in days				Period A		Total eggs		Fate
	A	B	C	D	Eggs laid		Laid	Hatched	
					% hatch				
B/K 1a	48	9	2	48	1260	98.3	1796	1359	Died, 107th day; dissected; no motile sperm present
B/K 2a	37	12	—	—	1058	99.0	1348	1182	Died, 49th day
B/K 16a	17	4	1	22	644	97.8	1374	675	Remated, 45th day; died, 52nd day, after laying fertile eggs
B/K 17a	21	24	—	—	713	98.5	1214	943	Died, 46th day
B/K 21a	30	15	46	6	1208	98.8	2276	1373	Died, 97th day; dissected; a few motile sperm present
B/K 25a	34	16	—	—	1133	99.0	1313	1273	Died, 50th day
B/K 1b	0	—	—	6	0	—	—	0	Remated, 7th day; discarded after laying fertile eggs
B/K 2b	14	10	0	9	423	100.0	1077	599	Died, 33rd day
B/K 16b	18	1	6	28	360	99.2	789	382	Died, 53rd day
B/K 17b	0	2	1	3	0	—	—	6	Remated, 7th day; discarded after laying fertile eggs
B/K 21b	13	2	16	43	465	98.5	1466	500	Remated, 75th day; died, 81st day, without laying further eggs
B/K 25b	13	1	4	20	367	99.5	1092	380	Died, 38th day
O 1a	19	2	20	67	544	59.5	1454	355	Died, 108th day; dissected; no motile sperm present
O 8a	9	7	36	47	261	42.9	740	250	Died, 99th day
O 10a	9	7	11	83	287	83.6	1342	408	Died, 110th day; dissected; no motile sperm present
O 14a	13	9	—	—	265	55.4	—	185	Escaped, 23rd day
O 15a	12	7	6	—	303	80.1	—	455	Remated, 26th day; discarded after laying fertile eggs
O 20a	0	—	3	8	0	—	—	1	Dissected, 12th day; a few motile sperm present
O 21a	9	4	6	6	332	68.0	—	273	Remated, 26th day; discarded after laying fertile eggs
O 22a	17	5	13	91	349	75.4	1274	325	Died, 126th day
O 27a	21	5	6	5	362	74.0	619	298	Died, 37th day
O 28a	3	2	17	21	99	45.5	983	92	Remated, 44th day; died, 51st day, without laying further eggs
O 1b	5	0	1	8	71	45.0	—	33	Dissected, 14th day; a few motile sperm present
O 8b	4	1	4	5	74	58.1	—	67	Dissected, 14th day; a few motile sperm present
O 10b	12	7	29	59	444	82.2	1817	538	Died, 107th day; dissected; no motile sperm present
O 14b	9	4	2	—	299	64.2	—	248	Crushed, 16th day
O 15b	17	7	10	6	334	68.5	535	274	Died, 40th day
O 20b	0	—	—	10	0	—	—	0	Dissected, 12th day; no motile sperm present
O 21b	11	5	18	74	317	81.4	2045	384	Died, 108th day; dissected; no motile sperm present
O 22b	6	2	12	5	127	76.4	—	151	Remated, 26th day; discarded after laying fertile eggs
O 27b	6	1	3	4	96	54.1	—	69	Dissected, 15th day; no motile sperm present
O 28b	0	—	—	6	0	—	—	0	Remated, 8th and 23rd days; discarded after laying fertile eggs

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whereas none of the fifty females kept by Clarke & Maynard Smith lived longer than 96 days. The difference is probably due to the slightly lower temperature in the present experiment.

(ii) *Rate of egg laying*

Table 2 gives the mean numbers of eggs laid at various ages; the following conclusions can be drawn:

Fertilized females with sperm in the receptacle lay approximately thirty eggs a day for the first 30 days after mating, after which they lay eggs at about half that rate. These are average figures; there are large fluctuations from day to day in the numbers of eggs laid by the same female. No female retained an adequate quantity of sperm for more than 52 days.

Table 2. *Rate of egg laying*

Days after mating ...		1	2, 3	4-10	11-17	18-24	25-31	32-38	39-45	46-52	53-59	60-73	74-94	95-101
Females laying fertile eggs	No. of females	—	27	21	15	8	5	5	5	1	—	—	—	—
	Mean daily lay	—	28.9	33.4	30.8	28.8	32.0	15.4	19.0	13.7	—	—	—	—
Females exhausted of sperm	No. of females	—	—	—	2	8	13	12	10	8	7	9	8	6
	Mean daily lay	—	—	—	37.7	29.6	22.5	8.9	9.8	13.4	16.6	9.4	5.5	3.7
Inadequately inseminated females	No. of females	—	7	7	—	—	—	—	—	—	—	—	—	—
	Mean daily lay	—	6.2	8.4	—	—	—	—	—	—	—	—	—	—
Females mated to testis-less males	No. of females	9	9	9	—	—	—	—	—	—	—	—	—	—
	Mean daily lay	0	1.2	4.2	—	—	—	—	—	—	—	—	—	—
Remated females aged 14-17 days	No. of females	2	4	2	—	—	—	—	—	—	—	—	—	—
	Mean daily lay	0	20.9	18.0	—	—	—	—	—	—	—	—	—	—
Remated females aged 30-52 days	No. of females	6	6	6	—	—	—	—	—	—	—	—	—	—
	Mean daily lay	0	0.5	19.3	—	—	—	—	—	—	—	—	—	—

The rate of egg laying by females exhausted of sperm is not greatly different, but the decline occurs slightly earlier. The rate continues to decline with age.

Inadequately inseminated females, or females mated to testis-less males, lay few eggs. Virgin females also lay a few eggs.

There is a delay in egg laying after remating, which is particularly marked in old females.

(iii) *Male fertility*

The total number of eggs laid by females which died a natural death varied from 536 to 2276, with a mean value of 1287. The number laid did not differ significantly for females mated to different types of males. However, the number of eggs which hatched varied greatly for different kinds of mating, as follows:

Type of male	First mating of male		Second mating of male	
	B/K	O	B/K	O
	Mean hatch			
	1134	264	301	176

Eggs laid by females mated to O males failed to hatch for two different reasons. During the initial period A a fraction of the eggs, approximately constant for a given mating, failed to hatch, probably because a fraction of the sperm produced by O males are inadequate to ensure the development of the zygote. The fraction of eggs hatching during this period is taken as a measure of male fertility, and varied from 0.429 to 0.836 for different matings to O males. In contrast, the fertility of matings to B/K males varied

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from 0.978 to 1.0. During the first 10 days, the ten females which were adequately inseminated by B/K males laid 3724 eggs, of which only fourteen failed to hatch, so that during this period 99.6% of the eggs hatched.

Thus the difference in fertility between O and B/K males is clear-cut. There are, however, differences between different O males, and between different matings by the same O male. Table 3 gives the fertilities of matings to O males, excluding ♂ O 20, which failed to produce sufficient sperm to make a measurement of fertility possible.

Table 3. *Variations in the fertility of O males in successive matings*

		Time after first mating				
	Mating	<i>a</i>	1 hr. <i>b</i>	7 days <i>c</i>	14 days <i>d</i>	21 days <i>e</i>
♂ O 1	Eggs counted	544	71	38	69	—
	% hatch	59.5	45.0	84.3	87.0	—
♂ O 8	Eggs counted	261	74	—	52	45
	% hatch	42.9	58.1	—	67.4	89.0
♂ O 10	Eggs counted	287	444	—	—	—
	% hatch	83.6	82.2	—	—	—
♂ O 14	Eggs counted	265	339	52	158	204
	% hatch	55.4	64.2	75.0	74.7	71.6
♂ O 15	Eggs counted	393	334	58	—	63
	% hatch	80.1	68.5	65.5	—	85.7
♂ O 21	Eggs counted	332	317	—	132	261
	% hatch	68.0	81.4	—	82.5	84.16
♂ O 22	Eggs counted	349	127	72	55	—
	% hatch	75.4	76.4	75.0	76.4	—
♂ O 27	Eggs counted	362	96	—	165	—
	% hatch	74.0	54.1	—	75.7	—
♂ O 28	Eggs counted	99	—	87	—	—
	% hatch	45.5	—	57.5	—	—

Considering first the original matings *a* and *b*, for the eight males which successfully inseminated both partners, it can be shown that there are significant differences between the fertility of a given male as measured by the first and second mating, a difference which may be in either direction. However, these differences are small compared to the differences between different males:

	χ^2	d.f.	$\chi^2/\text{d.f.}$	Variance ratio	<i>P</i>
Differences between the two matings by the same male	56	8	7.0	4.84	0.03
Differences between males	237	7	33.9		
Total	293	15			

Secondly, considering the later matings *c*, *d* and *e*, in some cases the fertility increased with age (e.g. males O 1 and O 8), and in others there was no change (e.g. male O 22). It is impossible at present to explain these variations in the fertilities of different matings to the same male. Consequently, although there are significant differences between the fertilities of different O males, it is difficult to give an accurate estimate of the fertility of any particular male.

The infertility of O males will account for a reduction of about 40% in the number of hatching eggs laid by a female mated to an O male as compared to a B/K male.

(iv) *Male fecundity*

During periods C and D many eggs fail to hatch because there are insufficient sperm in the receptacle to ensure fertilization. The data in Table 1 show that the duration of period A, during which the percentage hatch is constant, is usually greater for females mated to B/K males than to O males. The most likely explanation is that B/K males produce more sperm. An alternative explanation is that the sperm produced by B/K males survive for a longer time. The latter explanation, however, fails to account for the fact that the second female mated to a male, whether B/K or O, usually laid fertile eggs for a shorter time than did the first female to be mated. This would be expected if the differences in the duration of period A are due to differences in the number of sperm injected, but not if they are due to differences in the survival time of sperm.

It will therefore be assumed that we are concerned with a difference in the number of sperm produced; this will be called a difference in fecundity, as compared to the difference in fertility already discussed, which concerns the quality rather than the quantity of sperm.

A measure of male fecundity is given by the number of eggs laid by the female mated to that male before the percentage egg hatch starts to decline, i.e. by the number of eggs laid during period A. The mean values are:

Type of male	First mating		Second mating	
	B/K	O	B/K	O
Eggs laid during period A	1003	271	289	176

There is an approximately fourfold difference between the fecundities of B/K and O males as judged by their first matings; this accounts for most of the difference between the total numbers of hatching eggs laid by females mated by the two types of male. There is also a great reduction in the number of sperm produced in the second mating by both types of male.

4. MATING BEHAVIOUR

(a) *Normal courtship*

The courtship of *D. subobscura* has been described by Rendel (1945) and Spieth (1952), and in greater detail by Milani (1951a). In this species visual stimuli are the most important in influencing courtship behaviour; mating does not take place in the dark, and, unlike other species in the *obscura* group, males will attempt to copulate with wax models, i.e. lumps of wax about the size of a fly, provided the latter are moved in an appropriate manner (Milani, 1951b).

In the following description of normal courtship, which is based on my own observations, I have adopted Spieth's terms 'scissoring', 'tapping' and 'decamping'. The terms 'mating' and 'copulation' have been used synonymously, and imply that the male has mounted the female, and that the penis has been inserted into the female vagina. In practice it is difficult to observe the insertion of the penis, and it has been assumed to have taken place if the male has mounted in the normal position, and remained there for at least 1 min. Copulation usually lasts for from 10 to 30 min. By 'insemination' is meant that sperm has been injected into the vagina.

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An isolated male may make quick out-and-back movements of the wings, the movements often being repeated several times in succession. The frequency of such scissoring is greatly increased by the presence of other flies, and a male which scissors will usually court when opportunity arises.

When a male and female are paired, the male, as soon as he notices the female, approaches her and taps her with his front legs. The male then moves round so as to approach the female head-to-head, and protrudes his proboscis. The female then sidesteps rapidly to and fro, the male moving so as to maintain his position facing her; I shall refer to these movements as the 'dance'. A dance may be terminated without mating, if the female turns away from the male, or decamps, i.e. jumps or flies away. However, the female may stand still, extend her proboscis, and slightly part her wings (which in the closed position cover the abdomen), thus enabling the male to mount. In this case the male extends his wings stiffly sideways, the male and female probosces may touch, and the male then circles round behind the female and mounts.

The position of the male's wings during the dance varies, but usually they are slightly raised and extended, and are stretched stiffly sideways immediately prior to mounting, or if the female shows signs of decamping. Mounting and copulation occasionally occur immediately after the head-on approach of the male, without a dance. Males always protrude the proboscis, but there is much variation in the movements of the female proboscis. In consecutive courtships of three females in which the proboscis was observed, in one the female did not protrude the proboscis at all, in one the proboscis was protruded stiffly, came into contact with the male proboscis, and remained extended for several seconds after the male had mounted, and in the third courtship the female protruded and withdrew the proboscis rapidly several times immediately before the male mounted. Spieth observed that occasionally the labellar surfaces of the two individuals are brought into contact, and the proboscis moved alternately back and forth for about 5 sec., but I have not observed such behaviour.

When the first approaches of the male are unsuccessful, he may display movements of 'elaborate courtship' (Milani, 1951*a*), standing facing the female for several minutes at a time, not necessarily head-on, protruding the proboscis, tapping the female with his front legs, or rotating his middle pair of legs.

The above description agrees closely with that of Milani (1951*a*). Spieth (1952) emphasizes that *D. subobscura* is unique in that the male regularly postures while facing the female head-to-head, but does not mention the dance, which I have found to be the most striking feature of most courtships. Further, he states that, unlike Rendel (1945), he rarely observed scissoring by males. It seems therefore that the behaviour of the stock studied by Spieth differed in important respects from those studied by Rendel, Milani and myself; Spieth states that his stock was obtained from England, but gives no further details.

The movements of the dance could be interpreted as resulting from attempts by the female to avoid the male, and by the male to maintain his position facing her. This was my own view for a time, and may be part of the truth, but it is incomplete for two reasons. First, as will be described below, a female may occasionally step to and fro in front of a stationary male. Secondly, Milani's (1951*b*) results with wax models demonstrate that the rapid side-to-side movements of the female stimulate the male to attempt copulation. If a model is held stationary after first being moved rapidly from side to side, the male will

attempt to copulate with it; if such movements have not been made, the male will make the movements of elaborate courtship, but will not attempt copulation. It is perhaps not altogether unfamiliar to find that an incipient movement of escape or avoidance has been ritualized as a 'come-hither' signal.

(b) *Factors influencing the mating success of males*

Mating success is measured by the proportion of pairs observed to copulate within 1 hr. of being placed together in a vial. Since a number of pairs were observed simultaneously without magnification, copulation was the only event which was counted accurately. However, striking differences in behaviour in different experiments were noted.

If virgin B/K males, 3–5 days after eclosion, were paired with 7-day virgin females, copulation almost always occurred within 1 hr., provided that both males and females had been kept isolated for 24 hr. before pairing. Grouping together all such tests, mating success was 47/52; mating usually occurred within 15 min. of pairing. This high level of success provides a standard with which the success of pairs of different history or genotype can be compared.

(i) *Discouragement* of males*

Forty-four B/K males were paired with virgin females, but in this case the males had been kept in groups of five per vial until immediately before pairing. Mating success was only 12/44; in most cases no male courtship was observed. The thirty-two unmated pairs were then kept in the dark for 24 hr. Within 15 min. of returning to the light, 30/32 pairs had mated.

On another occasion, thirteen B/K males were paired with inseminated females. All the males courted, but were repelled by the females. The pairs were separated, and 3 hr. later the males were paired again with virgin females. Mating success was only 5/13, and in most of the remaining cases no male courtship was observed. The unmated pairs were kept in the dark for 24 hr., and mating success on return to the light was 7/8.

These two experiments suggest that males can be discouraged by courtship, either of other males or of inseminated females, in which the partner does not provide all the requisite stimuli, and which does not lead to mating. Such discouragement leads to a temporary cessation of courtship activities.

(ii) *Genetic differences between males*

Mating success of O males with virgin females was 19/30, or significantly lower than for B/K males. Mating success of inbred testis-less males was lower still. To obtain sufficient females which had mated with such males, it was sometimes necessary to test pairs repeatedly, with intervening periods in the dark. The results, for three successive batches of males, were:

7/14 after one trial, 3/4 after two trials, 8/16 after eight trials.

The low success of these inbred testis-less males is not mainly due to their lack of testes, nor to their *vermillion* eyes, as was shown by tests with outbred testis-less males. Mating success of outbred testis-less males was 11/12; ten of the males mated within 15 min.

* This word has been borrowed by Spurway (1955*b*) from Shaw's *Back to Methuselah*. She defines it as 'the waning of physiological capacity for an activity due to lack of consummation'.

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Observation of the inbred males suggested that the reasons for the lack of mating were different from those for discouraged B/K males. In most cases active courtship was seen, although mating did not follow. Prolonged dances, however, were rare. On two occasions, once with an inbred testis-less male, and once with an old O male (not included in the total of successes given above), the male approached the female head-on, and the female then stepped sideways to and fro several times in front of the stationary male before turning away. More usually, the males followed the sideways-stepping female, but lagged behind, and did not keep facing her, as did B/K males. Such dances usually ended with the female turning away. If, however, the female stood still and extended her proboscis, the male usually attempted to mount. Sometimes copulation was successful, but more often, particularly with inbred testis-less males, the male would fall on his back, or land too far forward or too far back on the female. In the latter case, the female would stand still with the wings partly extended for up to 10 sec. before kicking off the male. Such an unsuccessful attempt to mount a virgin female has only once been observed in a B/K male, although it is not infrequent with non-virgin females, which immediately kick off any male which attempts to mount. B/K males may also attempt to mount a virgin female after a dance, only to find that the female has decamped. The special feature of the unsuccessful attempts at mounting by inbred males is that the female may be standing still with the wings partly extended throughout the attempt.

Finally, inbred males which have courted a virgin female continuously but unsuccessfully for some time may approach the female from the side or from behind, and attempt to mount directly. Such behaviour probably results when a male has been courting, and has been receiving all the requisite stimuli to lead him to attempt mounting, but has not received the consummatory stimulus of copulation. The condition of such a male would be better termed desperation than discouragement. Direct attempts at mounting from the side or from behind were never successful, and were never seen from B/K males with virgin females, although sometimes attempted with non-virgin females.

It seems therefore that the lower mating success of inbred males is due, not so much to a lower intensity of courtship, as to lower athletic ability. This term is intended to include those capacities which in human beings contribute to athletic success, namely, physiologically efficient muscles, sense organs and neuromuscular co-ordination. The spirit is willing but the flesh is weak.

(iii) *Absence of sperm, or of testes*

The high mating success of outbred testis-less males shows that the presence of gonads is not necessary if a male is to court or to mate. Hollingsworth (1955) described a *D. subobscura* gynandromorph with a male head and female abdomen which courted like a male, orientating towards females and flicking the wings; unfortunately, the fly died before its behaviour when paired with males could be observed. However, Milani & Rivosecchi (1954) found that gynandromorphs in *Musca* showed courtship behaviour corresponding to the sex of their abdomens, and not of their heads.

The fact that a male has just mated does not cause any marked reduction in his sexual activity. Mating success of males paired within one hour of their first mating was:

B/K males: 12/14 in 15 min.
O males: 7/18 in 15 min., 12/18 in 1 hr.

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These values do not differ from those for virgin males. The data in § 3*b* show that in at least three cases the male had exhausted his sperm in the first mating, and could not inseminate the second female.

The results of experiments on the mating success of various types of males are summarized in Table 4.

Table 4. *Results of pairing males of various types with young B/K virgin females*

Type of male		Mating success	
		In 15 min	In 1 hr.
B/K males	Virgin	—	47/52
	1 hr. after first mating	12/14	—
O males	Virgin	—	19/30
	1 hr. after first mating	7/18	12/18
'Discouraged' B/K males	Kept in mass culture (males only)	—	12/44
	Previously paired with fertilized females	—	5/13
Outbred testis-less males		10/12	11/12
Inbred testis-less males		—	7/14
			3/4 (2 trials)
			8/16 (8 trials)

(c) *Factors influencing the readiness of females to mate*(i) *Fertilized females*

Thirteen B/K females which had been observed to mate with B/K males 6 days after eclosion were paired with young B/K males 7 days later. None of these pairs mated. On the approach of a male, fertilized females extrude the ovipositor and bend the abdomen towards the approaching male. Bastock & Manning (1955) have observed a similar movement in *D. melanogaster*, and find that it often leads to a temporary cessation of courtship by the male. The data in § 4*b* suggest that the same may be true in *D. subobscura*.

(ii) *Inadequately inseminated females*

Eight females which had been observed to mate were classified as inadequately inseminated, because they laid few eggs, of which few or none hatched. Three of these females were dissected; two contained very few motile sperm, and one no sperm. The other five females were paired again about 1 week later, four with B/K males and one with an O male. In all five cases remating occurred. The female remated to the O male again proved to have been inadequately inseminated; she was paired again with a B/K male, and mated for a third time after five unsuccessful trials.

These facts suggest that the stimulus which prevents fertilized females from remating is not the act of copulation, but of insemination. Only females which have received a quantity of sperm sufficient to enable them to lay fertile eggs refuse to remate.

This conclusion was confirmed by using testis-less males. Nine females which had been observed to mate with inbred testis-less males were paired with B/K males 6 days later, and mating took place in all cases. A similar result was obtained with eleven females, which had been observed to mate with outbred testis-less males; all eleven females remated with B/K males 2 days later. Whatever it is that is responsible for the change in the behaviour of fertilized females must be a product of the testes, and not of the accessory glands, which are present in testis-less males.

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Although females which have mated without being inseminated will remate 2 days later, the stimulus of mating may nevertheless prevent remating for a shorter period.

Eight B/K females were successfully mated to inbred testis-less males, and then paired with B/K males. Tests of 1 hr. in the light were then made at various intervals after the first mating, with intervening periods in the dark.

Six of the eight females were tested within 1 hr. of the first mating, and seven of them from 3 to 4 hr. after the first mating. In no case did a female remate, and during both tests the females were observed to extrude the ovipositor at approaching males. All eight females were retested approximately 24 hr. after the first mating, and in seven cases remating occurred within 5 min. of removal from the dark. The remaining female did not remate; she was tested again 2 days later, again without success. On dissection she proved to contain no sperm.

Thus, apart from one female whose behaviour was anomalous, mating without insemination prevented remating for a period greater than 4 hr. and less than 24 hr.

A control series of six B/K females were mated to B/K males, separated after mating, and paired again to fresh B/K males. The six pairs were retested on eight occasions at intervals from 1 hr. to 5 days after the first mating. Four of the six females did not remate. One remated 27 hr. after the first mating, during the fourth test, and one remated 44 hr. after, during the fifth test. Both matings were normal, no attempt being made to kick off the male. However, although two of the inseminated females did remate, the difference between the behaviour of inseminated and uninseminated females was quite sharp.

Hollingsworth (unpublished) has found that males will copulate with intersexes (females homozygous for the autosomal recessive *ix*), and that such intersexes will remate rapidly; the behaviour of intersexes, unlike that of females, is not altered by insemination.

(iv) *Females exhausted of sperm*

It has been shown that it is the act of insemination, not of copulation, which alters a female so that she does not remate after 24 hr. This suggested that the effective stimulus might be the presence of an adequate quantity of sperm in the receptacle. However, this is not the case, as is shown by the behaviour of exhausted females, i.e. of females which are laying eggs which do not hatch, although they have been observed to mate, and have previously laid many fertile eggs. Such females when dissected have proved to have no motile sperm in the receptacle, or more often a few motile sperm.

The results of pairing such females with young B/K males were:

Days after first mating	Mating success
26	3/9
30	0/13
37	0/13 (Two trials)
44	2/11 (Three trials)
50	0/8
75	1/9
88	0/8

These results contrast sharply with the behaviour of inadequately inseminated females, although the latter also may contain a few motile sperm. It seems therefore that adequate

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insemination is the effective stimulus which prevents remating for the rest of a female's life, and that its effect continues even when the female receptacle no longer contains an adequate quantity of sperm.

The results of experiments on the readiness of females to mate are summarized in Table 5.

Table 5. *Results of pairing females of various types with virgin B/K males*

Type of female	Mating success in 1 hr.
Virgin	47/52
Fertilized	0/13
Inadequately inseminated	5/5
Twice inadequately inseminated	1/1 (6 trials)
Previously mated to testis-less males: 1 hr. after first mating	0/6
4 hr. after first mating	0/7
24 hr. after first mating	7/8
2 days after first mating	11/11
1 week after first mating	9/9
Fertilized females exhausted of sperm	6 successes in 115 tests in- volving 19 females

5. DISCUSSION

(a) *Sexual selection*

Elaborations of courtship behaviour, and of associated structures, are common among animals. Since these features, both of behaviour and of structure, are the results of natural selection, it is reasonable to inquire what selective advantage has been conferred on the individuals possessing them. Different features of the courtship of an individual, and in particular of the courtship of the two sexes of a given species, may result from different selection pressures, especially where, as in *D. subobscura*, the males can mate many times and the females usually once only.

Three types of selective advantage, not necessarily mutually exclusive, have been suggested to account for these secondary sexual characters:

(i) Such characters ensure that an individual will mate with a member of its own, and not of a related species.

(ii) Such characters increase the breeding success of individuals possessing them. This may be because only such individuals can breed successfully; for example, robins which do not establish a territory do not breed (Lack, 1953), while the 'appeasement ceremonies' of female gulls may be necessary to elicit a sexual rather than an aggressive response from a male (Tinbergen & Moynihan, 1952). Alternatively, individuals with well-developed secondary sexual characters may mate more frequently, a possibility which arises mainly for the males of polygamous species, or such characters may ensure that an individual will mate at an appropriate time; for example, bitches during oestrus produce a characteristic odour.

(iii) Such characters result in an individual mating with one conspecific individual rather than another, the selection of a mate being such that the mating leaves a greater number of progeny. This requires that individuals possessing characters increasing their mating success are also fitter as parents, either because they are more fertile, or more efficient in caring for their young, or because their genotype is such that their progeny are on the average fitter than the progeny of other individuals.

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Darwin (1889) believed that such a correlation between success in mating and success as a parent, once mated, was necessary in most cases if secondary sexual characters were to evolve. He argued that where the numbers of the two sexes are equal, and in the absence of polygamy, all mature individuals can mate. Therefore success in sexual competition would not increase the fitness of a male, unless it ensured mating with a female of high fitness as a parent; conversely, the selection by a female of a male with well-developed secondary sexual characters would not increase her fitness, unless that male was also fit as a parent. Darwin thought that such correlations would in fact be found, because success both in sexual competition and as a parent would be features of the most vigorous individuals in a population.

It is doubtful whether Darwin was correct in thinking that correlations of this sort are a necessary condition for the evolution of secondary sexual characters in monogamous species, since even in such species a number of individuals of both sexes may fail to breed, so that selection of the kind discussed in § (ii) above can operate. They are certainly not necessary to account for the behaviour of males in polygynous species such as *D. subobscura*. Nevertheless, it seems that some features of the courtship of a female *D. subobscura* increase the likelihood of her mating with a male of high fertility, and may have evolved because of the advantage so conferred.

Considering first the results of the present experiments, it has been shown:

- (i) The mating success of outbred males is greater than that of inbred males.
- (ii) A female mated by an outbred male leaves approximately four times as many progeny as one mated by an inbred male.
- (iii) Females probably discriminate because males of high athletic ability follow with greater accuracy the rapid side-to-side movements of the female during the dance.

It follows that if the population contained only B/K and O males, the side-to-side movements of a female would confer selective advantage by increasing the likelihood of her mating with a fertile male. Whether selection of this type has been responsible for the evolution of the dance depends upon whether there is in wild populations a similar association between mating success, athletic ability and fertility. All are features of hybrid vigour, and the correlation between them has been exaggerated in the present work by comparing F_1 hybrids with inbred flies. However, differences in vigour, whether due to genetic, environmental or age differences, must exist in wild populations, and may well ensure sufficient correlation between the fertility and athletic ability of males to enable selection to operate in the way suggested.

The situation is probably a common one; its essential feature is that there is a correlation between the presence in an individual of characters responsible for mating success, and for fitness in other respects. If so, there will be a selective advantage for aspects of behaviour which have the effect of discriminating between different possible conspecific mates. The occurrence of this type of Darwinian sexual selection is of some interest. Recent discussions of the evolution of courtship behaviour (e.g. Mayr, 1948; Dobzhansky, 1951) have tended to emphasize its importance in preventing interspecific matings. That selection can produce this result has been demonstrated by Koopman (1950). However, Spurway (1955*a*) argued that the most generalized selection pressure influencing courtship may be to introduce a non-random element in fertilization. The present results tend to support this view.

(b) *Insemination reaction*

It has been shown that it is the stimulus of insemination, and not of copulation, which reduces the readiness of a female to remate, and which causes an increase in the rate of egg laying. In some species of *Drosophila* insemination is followed by a reaction between the ejaculate and the walls of the vagina (Patterson, 1946; Patterson & Stone, 1952), and there may be some connexion between this reaction and the ensuing change in behaviour.

After insemination, the vaginal pouch becomes swollen with an opaque reaction mass, which in intraspecific matings remains fluid and is ejected after from 6 to 12 hr. In interspecific matings, the reaction mass may become crystalline, and persist for a week or more. In such cases the reaction reduces the number of sperm which reach the ventral receptacle, interferes with fertilization of the eggs, and may cause cytolysis of the eggs. Since the reaction may occur after insemination by sterile hybrid males, it does not depend on the presence of motile sperm, but the work of Lee (1950), who injected various substances into the vagina, suggests that the reaction is induced by some product of the testes, and not of the accessory glands.

A visible reaction occurs in some but not all species in the genus (Wheeler, 1947). Rendel (1943) observed a substantial sperm plug, which sometimes interfered with egg laying, in *D. subobscura* females inseminated by *D. pseudoobscura* males. However, Mr M. J. Hollingsworth (unpublished) finds that there is no visible reaction in intraspecific matings of *subobscura*. A reaction may nevertheless take place with similar physiological effects, but without producing a visible reaction mass. Even so, his observation contradicts the statement of Patterson & Stone that 'it is known that females in which the reaction does not occur will remate two or more times, often at frequent intervals'.

Patterson & Stone suggest various possible 'functions' of the insemination reaction; however, their discussion appears to confuse the possible effects of the reaction with the possible selective advantage conferred by it. The possible functions suggested are:

(i) The reaction prepares the reproductive tract for the fertilization mechanism, and for egg laying. This may well be the case, and if so the reaction has a selective advantage in intraspecific matings.

(ii) The reaction reduces gene exchange between species. This is almost certainly a *result* of the prolonged reaction in interspecific matings. However, a female which shows such a prolonged reaction is thereby sterilized, or at least her fertility is reduced. The reaction cannot increase her fitness, and the prolonged reaction in interspecific matings cannot therefore be the direct result of selection.

(iii) The reaction prevents remating. Patterson & Stone argue that this would be of selective advantage in two ways. First, 'it would more nearly ensure that all females are fertilised within a species'. This does not seem to follow. Secondly, 'the prolonged reaction in interspecific matings would prevent remating of the female to males of her own species, and so select for more complete sexual isolation'. Now if females which mate with males of another species are effectively sterilized, this will increase the selective advantage of any behaviour reducing the frequency with which a female accepts such a male. It does not follow that the prolonged reaction in interspecific matings is itself an advantage to the female, or that it is selected for.

(iv) Finally, a mutation which would cause the semen of a male to induce this reaction would spread, as a male with such a mutation could follow other males and remate with

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a female, whereas normal males could not remate with females after such a male. This is an ingenious argument, but it seems unlikely that such a mutation could spread unless the reaction conferred some advantage on the reacting female as well as on the inducing male.

It seems therefore that the insemination reaction must be a selective advantage to one, and probably to both partners in intraspecific matings, but that the nature of this advantage is uncertain. The prolonged reaction in interspecific matings both reduces the gene flow between species, and imposes additional selective advantage on behavioural isolating mechanisms. However, the prolonged reaction does not itself confer advantages which have been selected for, any more than hybrid inviability or infertility confer such advantages.

Whether the unwillingness of *subobscura* females to remate is due to a reaction analogous to the insemination reaction or to some other cause, it results, in laboratory conditions, in a long post-reproductive life, and in some reduction in the number of fertile eggs laid. However, it is unlikely that either of these results ensues in the wild. First, relatively few wild females may live to such an advanced age, and, more important, few wild females can lay eggs, and so use up sperm, at such a high rate, since this would imply the continuous presence of suitable oviposition sites. Little is known either of the longevity of females in the wild, or of where the eggs are laid. However, a number of females caught in November by Dr Dyson-Hudson and subsequently kept by the writer were judged to be old, both from their dark cuticles and damaged wings, and from the fact that they laid few eggs and died soon after capture. Only nine of some twenty females laid eggs in test vials, but all the eggs laid hatched. These facts suggest that the long post-reproductive life may be a laboratory artifact.

6. SUMMARY

The eggs laid by outbred females have been collected from the time of mating until the death of the females, and the proportion of eggs hatching recorded. Initially over 99% of the eggs hatch after mating to outbred males; after about 30 days the quantity of sperm in the ventral receptacle is inadequate to ensure fertilization, and few or no eggs hatch. After mating to inbred males, the initial hatch varied from 43 to 84%, and a sharp decline in the proportion hatching occurred after about 10 days. Thus inbred males are inferior both in the quality and quantity of sperm produced.

The effects on fertility of the age of the male, and of previous matings by the male, have been studied.

Females which have once been inseminated rarely remate, even when tested 2–3 months later, when their receptacles have been exhausted of sperm. However, females which have been observed to mate with testis-less males, and which therefore have not been inseminated, will remate 24 hr. later.

The mating success of inbred males is lower than that of outbred males. Observation suggests that this is because inbred males do not follow the rapid side-to-side courtship movements of the female, and that this failure of inbred males is due to low athletic ability, and not to low sexual drive. If a similar correlation exists in wild populations between the athletic ability and the fertility of males, the courtship movements of a female will confer selective advantage in ensuring that she mates with a fertile male.

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