EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. IV. MODIFICATION OF THE DEGREE OF ISOLATION BETWEEN DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS AND OF SEXUAL PREFERENCES IN DROSOPHILA PROSALTANS

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Sexually active animals find their potential mates and recognize them as belonging to the same species with the aid of stimuli that are but poorly known. In birds, it has been shown that species recognition may be either strictly innate or conditioned through experience (for a short summary of the literature see Cushing[1]). Conditioning seems to play a major rôle particularly in species with highly developed parental care. On the other hand, innate mechanisms control the recognition of potential mates of the same species in birds that are not raised by their own parents, such as cowbirds, parasitic cuckoos and megapodes. The same seems to be true for most of the lower vertebrates and invertebrates. It is, however, very little known to what extent the functioning of the innate patterns may be influenced by conditioning and by other extrinsic factors. The experiments described below were devised to explore the possibilities in this field.

Material and Methods.—For most of the experiments an orange-eyed mutant strain of *Drosophila pseudoobscura* Frolova descended from flies collected at Piñon Flats, San Jacinto Mountains, California, and a wild strain of *Drosophila persimilis* Dobzhansky and Epling from Stony Creek, north of the Sequoia National Park, California, were used. For some of the experiments strains of *Drosophila prosaltans* Duda from Chilpancingo and Zopilote Canyon, Mexico, and from Belem, Iporanga, and Bertioga, Brazil, were employed (concerning these strains see Dobzhansky and Streisinger[2]).

The two species, *D. pseudoobscura* and *D. persimilis*, are almost indistinguishable morphologically, although Reed, Williams and Chadwick[3] were able to discriminate the strains at their disposal with the aid of an ingeniously contrived ratio of thorax volume to the product of wing area times the cubed wing length. *D. persimilis* was formerly known as "*D. pseudoobscura* race B." The irrationality of this designation became progressively more clear with the accumulation of data showing that these species are distinct genetic systems; their separation is fully maintained in nature despite the broad overlapping of their distribution areas.[4] Too great an emphasis on morphological distinctions as species criteria leads to results that are plainly untenable. One would have to break living mankind into five species that are not at all isolated reproductively,[5] and yet consider as

single "species" some groups of clearly separate species of Drosophila.[6]

Except when stated otherwise, the experimental procedure was the same as described in the preceding parts of this series.[2, 7] Batches of ten freshly hatched females of each of two species or strains to be tested were placed with ten males of one of these species in vials with food. After 4 to 7 days, depending on species and the particular experiment, the females were dissected and their sperm receptacles and spermathecae examined for sperm. The amounts of sperm present in an inseminated female vary greatly, particularly in heterogamic crosses: sometimes the ventral receptacle is tightly filled with sperm, sometimes loosely, and sometimes only a few moving spermatozoa are found. No attempt was made to record the various degrees of insemination; so long as any sperm was found, the fly was recorded as inseminated. In only a single *D. pseudoobscura* female, sperm was found in the spermatheca but none in the receptacle. The stock bottles were kept mostly at room temperature, and the experimental vials in an incubator at $24^1/_2$°C. Since the experiments lasted from May to November, "room temperature" varied considerably, and this may be a source of error in some of the experiments.

Sexual isolation between *D. pseudoobscura* and *D. persimilis* was first discovered by Lancefield[8] and subsequently studied by Boche,[9] but the data of the last-named investigator have never been published. We found the isolation to be much stronger when *D. pseudoobscura* than when *D. persimilis* males are used; it must, however, be noted that our experiments concern a single strain of each species, and that other strains may quite conceivably behave differently. Such differences between strains of *D. pseudoobscura* and *D. persimilis* with respect to sexual isolation from a third species, namely, *D. miranda*, are, indeed, known.[10]

Mixed Cultures.—Specific smells may be very important components of isolating mechanisms in animals with a highly developed olfactory sense. Experiments were, therefore, arranged to test whether or not there was a difference in the degree of sexual isolation between *D. pseudoobscura* and *D. persimilis* when these flies are raised in separate culture bottles or together in the same bottle. Fertilized females of the two species were placed together, but without males, in the same culture bottle, and transferred to fresh bottles at about 24-hour intervals. The larvae of both species grew up together in the same culture medium. The flies of the two species were separated after hatching before any copulation could occur. Sets of 10 females of both species were then confined with 10 males of one or the other species in vials with food, whereupon the females were dissected and their seminal receptacles were examined for sperm. As a control, similar tests were made using flies of the two species which developed in separate bottles.

It can be seen from table 1 that raising flies of the two species in the same culture medium does not lower the sexual isolation between them; curiously

enough, the results seem to indicate, if anything, the opposite. It probably occurs in nature not infrequently that larvae of the sympatric species *D. pseudoobscura* and *D. persimilis* grow up in the same food medium, and it is obviously of survival value to both species that the mixing of the smells of their larvae does not lead to lowering of isolation between the two species.

TABLE 1

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN CROSSES IN WHICH *D. pseudoobscura* AND *D. persimilis* FLIES WERE RAISED TOGETHER IN THE SAME BOTTLE OR IN DIFFERENT BOTTLES

MALE	RAISED	HOMOGAMIC *n*	HOMOGAMIC %	HETEROGAMIC *n*	HETEROGAMIC %	χ^2	ISOLATION INDEX
pseudoobscura	separately	82	87.8	83	9.6	100.7	0.80
pseudoobscura	together	145	89.6	140	0.7	226.6	0.98
persimilis	separately	127	63.8	119	22.7	42.5	0.48
persimilis	together (May–June)	41	73.2	41	2.4	43.6	0.94
persimilis	together (July)	82	76.5	89	32.9	31.2	0.40

Conditioning.—A set of *D. pseudoobscura* males was divided in two parts; some males were kept for 8–15 days in regular culture bottles with an excess of females of their own species ("pro-conditioned"), and others for the same length of time with females of *D. persimilis* ("counter-conditioned"). Similarly, some *D. persimilis* males were "pro-conditioned" and others were "counter-conditioned." Groups of 10 males were, then, confined with 10 freshly hatched females of each of the two species in vials with food; the females were dissected and examined for sperm. In "control" experiments freshly hatched males were confined with freshly hatched females. The results are summarized in table 2.

TABLE 2

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN CROSSES OF *D. pseudoobscura* AND *D. persimilis*

MALES	HOMOGAMIC *n*	HOMOGAMIC %	HETEROGAMIC *n*	HETEROGAMIC %	χ^2	ISOLATION INDEX
pseudoobscura control	82	87.8	83	9.6	100.7	0.80
pseudoobscura pro-conditioned	97	81.4	93	0.0	129.0	1.00
pseudoobscura counter-conditioned	115	88.7	122	1.6	182.7	0.96
persimilis control	127	63.8	119	22.7	42.5	0.48
persimilis pro-conditioned	32	56.3	37	13.5	13.9	0.69
persimilis counter-conditioned	47	87.2	52	38.5	25.0	0.39

The results are inconclusive as far as *D. pseudoobscura* males are concerned. There seems to be less isolation among the controls than among the counter-conditioned flies. However, the "control" experiment employed freshly hatched males while in the conditioning experiments males were 8–15 days old. Furthermore, most of the control experiments were made in May while the conditioning experiments were made in July. In

the case of *D. persimilis* males the conditioning appears to be effective. Males that had been conditioned with their own females show a higher isolation index (0.69) than the controls (0.48) or males conditioned with *D. pseudoobscura* females (0.39). The χ^2 of the difference between control and pro-conditioned flies is 2.08 (for two degrees of freedom $P > 0.20$); the χ^2 of the difference between control and counter-conditioned flies is 13.63 ($P < 0.01$). The effects of counter-conditioning are more significant than those of pro-conditioning.

Light.—Philip, Rendel, Spurway and Haldane[11] have stated that *D. subobscura*, a European relative of *D. pseudoobscura* and *D. persimilis*, does not mate in the absence of light, and that normal females of this species kick off mutant males with a yellow body color. Although morphological differences between *D. pseudoobscura* and *D. persimilis*, as well as those between strains of *D. prosaltans*, are very slight, the possibility that visual stimuli are involved in mate recognition is not excluded. To test this, vials were prepared containing two kinds of females and a single kind of males.

TABLE 3

MATE DISCRIMINATION IN THE LIGHT AND IN THE DARK

LIGHT OR DARK	FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	ISOLATION INDEX
Light	pseudoobscura, persimilis	pseudoobscura	40	80.0	40	7.5	0.83
Dark	pseudoobscura, persimilis	pseudoobscura	60	80.0	69	2.9	0.93
Light	pseudoobscura, persimilis	persimilis	100	78.0	100	40.0	0.32
Dark	pseudoobscura, persimilis	persimilis	100	93.0	100	60.0	0.22
Light	prosaltans-A, prosaltans-D	prosaltans-A	70	82.9	65	3.1	0.93
Dark	prosaltans-A, prosaltans-D	prosaltans-A	69	46.4	68	1.5	0.94
Light	prosaltans-B, prosaltans-C	prosaltans-B	68	39.7	68	2.9	0.86
Dark	prosaltans-B, prosaltans-C	prosaltans-B	59	18.6	57	0.0	1.00

Some of the vials prepared on each of the days during which the experiments lasted were placed in an opaque box and the others on the top of the same box; the box was exposed to daylight but protected from direct sunlight. The temperature varied in the environment, but it was obviously very similar inside and outside the box. Females of the "dark series" were dissected soon after being removed from the box. The results are summarized in table 3; in this table the strains of *D. prosaltans* coming from Chilpancingo, Zopilote, Belem and Bertioga are denoted "prosaltans-A," B, C and D, respectively.

It is evident that in *D. pseudoobscura*, *D. persimilis* and *D. prosaltans* the mate discrimination is not greatly influenced by the presence or absence of light. In *D. prosaltans* the light has, however, an obvious influence on the total number of inseminations taking place within a given time interval; a significantly greater number of matings takes place in the vials exposed to light than in those kept in the dark. The data for *D. persimilis*,

if taken at their face value, would indicate an opposite effect of light, but the differences observed are in need of confirmation.

The Role of the Wings.—When a courting male of *D. pseudoobscura* or *D. persimilis* pursues a female he spreads and vibrates his wings. The pitch of vibration may be correlated with the wing surface[3] which is larger in the latter than in the former species. If females exercise the choice, it is possible that they are helped in recognition of the males by the pitch of the wing vibration. If so, the females should have difficulties in recognizing wingless males, and the isolation index should drop. Actually, the opposite happened when wingless *D. persimilis* males were confined with winged *D. persimilis* and *D. pseudoobscura* females—the isolation index became higher (table 4). However, the point when around 50 per cent of the females were inseminated was not reached after 4–5 days' exposure, as with normal males, but only after 8 days. Females seem to recognize the species of wingless males as readily as of normal ones, but either succeed better in avoiding insemination by not conspecific wingless males or are less easily excited into a receptive state.

TABLE 4

INSEMINATION RECORDS OF *D. persimilis* AND *D. pseudoobscura* FEMALES BY WINGLESS *D. persimilis* MALES

HOMOGAMIC		HETEROGAMIC			ISOLATION
n	%	*n*	%	χ^2	INDEX
78	65.4	70	12.8	42.8	0.67

Experiments with wingless females and normal males resulted in isolation indices which are practically identical with the control experiments. This is important if considered in conjunction with the observation that non-receptive females often flick off with their wings males which attempt to mount them. Non-receptive wingless females seem to be equally capable of avoiding males.

Sexual excitement.—Dobzhansky and Koller,[10] working with *D. pseudoobscura* and *D. miranda*, obtained an indication that males aged in the absence of females are less efficient in discriminating between their own and foreign females than males pro-conditioned with their own females. If significant, this result may be due either to sexual excitement of the males aged without females or to the pro-conditioning of the other group of males. We have kept males and females of *D. pseudoobscura*, *D. persimilis*, and of four strains of *D. prosaltans* in isolation from individuals of the opposite sex but with abundant food for approximately seven days, whereupon these fully mature flies were placed together in the same vial, always avoiding etherization of the flies. As in the earlier experiments, one kind of males and two kinds of females were placed in each vial. The difference between this technique and that of Dobzhansky and Koller[10] lies in that in the experi-

ments under consideration both males and females were aged in isolation, while in the latter experiments only the males were so aged. Courting and copulating pairs can be seen in the vials within a few minutes after the flies are placed together, and in from one to four hours approximately half of all females are found to be inseminated. If freshly hatched flies are used, it takes from four to five days for half of the females to become inseminated, and relatively few copulating pairs are seen in the vials at any one time. The results of the experiments are summarized in table 5. The

TABLE 5

MATE DISCRIMINATION IN INDIVIDUALS AGED IN ISOLATION FROM THE OPPOSITE SEX

FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	χ^2	ISOLATION INDEX
pseudoobscura, persimilis	pseudoobscura	40	70.0	37	5.4	20.53	0.85
pseudoobscura, persimilis	persimilis	43	67.4	56	7.1	26.65	0.81
prosaltans-A, prosaltans-D	prosaltans-A	63	93.6	74	23.0	30.50	0.61
prosaltans-B, prosaltans-E	prosaltans-E	38	2.6	43	67.4	22.63	−0.92

Chilpancingo, Zopilote, Bertioga and Iporanga strains of *D. prosaltans* are referred to in this table as "prosaltans-A," B, D and E, respectively. Frequencies of the homogamic and heterogamic fertilizations in mixtures of *D. pseudoobscura* and *D. persimilis* shown in table 2 may be taken as control values for comparison with the data in table 5, although these experiments have not been performed simultaneously. For insemination records in *D. prosaltans* see Dobzhansky and Streisinger.[2]

Examination of table 5 shows that aging in the absence of individuals of the opposite sex fails to change the degree of sexual isolation when *D. pseudoobscura* males are used; with *D. persimilis* males, such aging leads even to a strengthening of the isolation, although more data are needed to establish this point. Aged prosaltans-A (Chilpancingo) males gave a somewhat lower isolation index than was obtained with males placed together with their prospective mates shortly after their hatching from the pupae; males of prosaltans-E (Iporanga strain) prefer B (Zopilote) females to their own, and this preference seems to be enhanced by aging in the absence of mates.

Temperature.—Flies raised at room temperature were placed in vials soon after their hatching from pupae, and the vials with the flies were kept at $24^1/_2°$, 21°, 18° and $16^1/_2°$C. for as long as necessary to obtain insemination of about half of the females. This takes 4–5 days at the higher and 7–9 days at the lower temperatures. The results are summarized in table 6. "Prosaltans-A," B, C and D are, in this table, the Chilpancingo, Zopilote, Belem and Bertioga strains of *D. prosaltans*, respectively.

The behavior of *D. pseudoobscura* and *D. prosaltans* flies is about the same at all the temperatures tried. *D. persimilis* shows clear sexual isola-

tion from *D. pseudoobscura* at the higher temperatures, but at 18° and 16½° *D. persimilis* males seem to discriminate against females of their own species in favor of those of *D. pseudoobscura*. This is particularly astonishing because *D. persimilis* is, on the whole, confined in nature to cooler habitats than *D. pseudoobscura*. It may be that females of *D. persimilis* become sexually receptive only very slowly at temperatures of 18°C. and lower, so that most *D. persimilis* females in the low temperature experiments were simply unavailable for insemination. To test this possibility, *D. persimilis* females and males, and *D. pseudoobscura* females, were aged for 10 days at 16½°C., and placed together, at the same temperature, for about 24

TABLE 6

MATE DISCRIMINATION AT DIFFERENT TEMPERATURES

t°	FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	χ^2	ISOLATION INDEX
24½°	pseudoobscura, persimilis	pseudoobscura	30	83.3	28	3.6	20.4	0.92
18°	pseudoobscura, persimilis	pseudoobscura	21	85.7	18	0.0	15.4	1.00
16½°	pseudoobscura, persimilis	pseudoobscura	42	92.9	40	12.5	24.2	0.76
24½°	pseudoobscura, persimilis	persimilis	65	93.8	64	39.1	14.6	0.41
21°	pseudoobscura, persimilis	persimilis	56	53.6	63	12.7	15.4	0.62
18°	pseudoobscura, persimilis	persimilis	21	4.8	20	55.0	8.7	−0.84
16½°	pseudoobscura, persimilis	persimilis	86	32.6	90	52.2	4.0	−0.23
24½°	prosaltans-A, prosaltans-C	prosaltans-A	59	86.4	58	8.6	37.8	0.82
24½°	prosaltans-B, prosaltans-D	prosaltans-B	58	74.1	58	8.6	30.1	0.79
16½°	prosaltans-A, prosaltans-C	prosaltans-A	77	90.9	75	2.3	62.3	0.94
16½°	prosaltans-B, prosaltans-C	prosaltans-B	84	44.0	85	4.7	14.6	0.81

hours. Dissection of the females showed that 59.4% of the 106 *D. persimilis*, and 44.6% of the 92 *pseudoobscura* females were inseminated. This gives a non-significant positive isolation index of 0.14. Sexual isolation between *D. pseudoobscura* and *D. persimilis* appears, then, to be weaker at lower than at higher temperatures if males of *D. persimilis* are used.

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*DOMINANCE MODIFICATION AND PHYSIOLOGICAL EFFECTS OF GENES**

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Several years ago[1] there was found in the house mouse a mutation with several striking effects including absence or shortening of the tail, absence or abnormality of one or both kidneys, absence of external anus and genital aperture and abnormalities of other parts of the urogenital system. In the stock in which it occurred this whole syndrome of effects behaved as a unit and showed simple segregation from normal. The mutation acted as a lethal, all homozygotes *Sd Sd* being tailless and dying shortly after birth, all showing imperforate anus and absence of both kidneys. Heterozygotes showed a lesser expression of these defects, having short tails, and less severe urogenital malformations. The mutation in the original stock therefore acted as a dominant in respect to its effect on the tail, as recessive or nearly so in its lethal effect, and as incompletely dominant in its effect on urogenital development.

When the *Sd* mutation was removed from the stock in which it originally occurred and was transferred by a series of successive backcrosses to another inbred normal stock, the tail length of heterozygotes progressively decreased until after five backcross generations nearly all were tailless, while the viability of the heterozygotes decreased, due to the greater effect of *Sd* on the urogenital system.[1] About 90 per cent of all *Sd*+ animals at birth had abnormal kidneys.[2] The dominance of *Sd* on tail length appeared to have been increased by the genetic constitution of the new stock while the lethal effect appeared also to have become partially dominant. There was no evidence of necessary connection between the effect upon tail length and upon viability.

Since the above observations were published we have transferred the *Sd* mutation to two other normal-tailed inbred stocks by repeated backcrossing. In one of these stocks (identified as *m*) the tail length of heterozygotes increased, and the proportion of tailless animals among the heterozygotes decreased. In the F_1, BC_1 and BC_2 generations the cross of *Sd*+ by normal *m* produced 142 normal, 90 short-tailed and 25 tailless; while in BC_3 and BC_4 the comparable figures are 39 normal, 23 short and no tailless. The