EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. V. THE EFFECT OF VARYING PROPORTIONS OF DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS ON THE FREQUENCY OF INSEMINATION IN MIXED POPULATIONS

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Introduction.—When females of *Drosophila pseudoobscura* and *Drosophila persimilis* are placed together with males of one of these species, it is usually found that a greater proportion of the conspecific than of the alien females are inseminated. The nature of the stimuli that lead to this result is still obscure, although Mayr and Dobzhansky[1] have shown that the degree of the preference varies depending upon the history of the individual flies involved and upon the environment. The experiments to be reported in the present article are concerned with the effects of the relative numbers of the conspecific and of the alien females available to the males on the frequencies of homogamic and heterogamic matings. Perhaps the simplest of the many hypotheses that may be constructed about the matings which take place in mixed populations is that when a female and a male meet, there is a certain definite probability that they will mate, depending on their respective species but not on what other males and females may be found in the same medium. On the other hand, it is also possible that the flies are stimulated by the proximity of conspecific individuals of the opposite sex in such a manner that they become more receptive to mating with alien as well as with conspecific partners than they would otherwise be. Still another possibility is that only homogamic matings take place while conspecific individuals of the opposite sex are available, and that heterogamic matings occur only, or predominantly, when the possibility of homogamic mating is excluded or remote. The above possibilities are not necessarily mutually exclusive, and various intermediate situations may be encountered. It is also fully conceivable that different pairs of species and races might behave quite differently in this respect.

Methods and Procedure.—The orange strain of *D. pseudoobscura* and the Stony Creek strain of *D. persimilis* were used in all experiments.[1] In the main body of seven experiments, 10 freshly hatched *D. persimilis* males were placed in each vial together with the following freshly hatched females: (1) 10 *persimilis*, (2) 10 *pseudoobscura*, (3) 10 *persimilis* and 10 *pseudoobscura*, (4) 20 *persimilis* and 10 *pseudoobscura*, (5) 40 *persimilis* and 10 *pseudoobscura*, (6) 10 *persimilis* and 20 *pseudoobscura*, and (7) 10 *persimilis* and 40 *pseudoobscura*. In other words, only *D. persimilis* males were used in all vials and their numbers were always ten per vial; the numbers and the species of the females were varied. The vials were kept in an incubator at $25^1/_2$°C. for approximately 4 days, whereupon the females were dissected and the presence or absence of sperm in their seminal receptacles was determined by microscopic examination. These experiments were done in September–October, 1944, with the assistance of Mr. George Streisinger, whose help we wish to acknowledge. The results are summarized as experiments 1 to 7 in table 1.

TABLE 1

NUMBERS OF INSEMINATED FEMALES OBSERVED WHEN 10 *D. persimilis* MALES WERE PLACED WITH n_c *D. persimilis* FEMALES AND n_a *D. pseudoobscura* FEMALES

			D. persimilis			*D. pseudoobscura*				
EXPT. NUMBER	NO. OF CONSPECIFIC PER VIAL	NO. OF ALIEN PER VIAL	FERTILIZED	UNFERTILIZED	TOTAL	FERTILIZED	UNFERTILIZED	TOTAL	ISOLATION INDEX	ISOLATION RATIO
x	n_{cx}	n_{ax}	F_{cx}	$N_{cx}-F_{cx}$	N_{cx}	F_{ax}	$N_{ax}-F_{ax}$	N_{ax}	b_x	g_x
			....*	....						
1	10	0	115*	28	143					
			80.41*	19.59						
						...	....			
2	0	10				28	125	153		
						18.30	81.70			
			143.67	34.33		27.34	145.66			
3	10	10	141	37	178	39	134	173	0.5569	0.2846
			79.21	20.79		22.54	77.46			
			149.68	41.33		14.27	78.73			
4	20	10	150	41	191	13	80	93	0.6978	0.1780
			78.53	21.47		13.98	86.02			
			204.67	168.33		9.67	80.33			
5	40	10	204	169	373	11	79	90	0.6347	0.2235
			54.69	45.31		12.22	87.78			
			57.03	44.97		22.99	187.01			
6	10	20	60	42	102	17	193	210	0.7581	0.1376
			58.82	41.18		8.10	91.90			
			87.72	41.28		70.17	456.83			
7	10	40	90	39	129	64	463	527	0.7035	0.1741
			69.77	30.23		12.14	87.86			
						...	....			
8	0	10				49	80	129		
						37.98	62.02			
			....	....		...	....			
9	10	10	122	6	128	67	63	130		
			95.31	4.69		51.54	48.46			

* For each experiment, the top line gives the "expected number" of flies on the basis of H2′ (see text), the middle line gives the observed numbers, and the bottom line gives the observed percentages.

Additional data were secured in April, 1945, with the aid of a slightly different method. Males and females of *D. persimilis* and *D. pseudoobscura* were aged in the absence of individuals of the opposite sex for a week to ten days, whereupon 10 *persimilis* males were placed in vials overnight with 10 *pseudoobscura* females or with 10 *pseudoobscura* and 10 *persimilis* females. (Experiments 8 and 9 in table 1.)

Similar experiments were performed on two geographic strains of *D. prosaltans*, but these will be discussed in a separate section.

When freshly hatched females and males were kept together for 3–4 days before dissection, the results obtained in different vials of the same experiment frequently showed statistically significant heterogeneity, while the experiments with the aged flies did not. The heterogeneity is, of course, a complicating factor in the analysis of the data. There are several probable sources of this heterogeneity. Since dissection and examination of the sperm receptacles is a rather laborious operation, females and males in some vials were kept together somewhat longer than in others. Some flies came from culture bottles in the early and others in late stages of hatching. Since the experiments of a given series extended for about two months, some variation in the food and other environmental factors may have occurred.

Analysis.—Throughout this discussion we will assume that there are 10 males of a given species or strain present in a standard vial, along with n_c conspecific[2] females and n_a alien females. Of these f_c and f_a, respectively, will be fertilized. Let N_c equal the sum of the n_c for a given experiment (i.e., N_c is the total number of conspecific females in all the vials of a given composition), and let N_a, F_c and F_a be the corresponding sums for n_a, f_c and f_a.

If π_c is the probability that any particular conspecific female will be inseminated during the course of the experiment, then π_c will be some function of n_c, n_a and of t, the time that the flies are left together. We denote this function by $\pi_c(n_c, n_a, t)$. We will include in t also such factors as temperature, age of the flies, food, etc., so that t represents what may be called the "physiological time." Similarly $\pi_a(n_c, n_a, t)$ is the probability that an alien female will be inseminated. Evidently π_c and π_a approach zero as the number of females per vial becomes large, since there must be some upper limit to the number of females a male can inseminate. Because of the variability from vial to vial of the per cent fertilized, as above discussed, the data do not give very precise information on the form of the functions π_c and π_a. However, from examination of the results for the individual vials it is clear that there is no very great variation in π_c over the range of values of n_c and n_a used, with the possible exception of a decrease in π_c in the experiment with 40 conspecific and 10 alien females. Apparently the decrease in percentage of fertilization does not become marked until considerably higher densities of females are reached than were used here. Since any analytic function is approximately linear for small changes in the variables, we may suppose that π_c depends on some linear combination of n_c and n_a when n_c and n_a do not vary too widely. We may represent this combination as $n' = dn_c + en_a$ where d and e are constants. If, now, $e/d = 0$, π_c depends only on the number of conspecific females, while if $e/d = 1$ it depends only on the total number of females. Since π_c apparently decreases for $n_c = 40$, $n_a = 10$ and not for $n_c = 10$, $n_a = 40$, e/d seems to be a positive fraction less than one.

Stalker[3] has proposed an "isolation index" $\beta = (\pi_c - \pi_a)/(\pi_c + \pi_a)$. An equivalent but somewhat simpler index is $\gamma = \pi_a/\pi_c$. They may be interchanged by the formulas $\beta = (1 - \gamma)/(1 + \gamma)$ and $\gamma = (1 - \beta)/(1 + \beta)$. If only homogamic matings occur, $\beta = 1$ and $\gamma = 0$; if there is no discrimination, $\beta = 0$ and $\gamma = 1$; and if only heterogamic matings occur, $\beta = -1$ and $\gamma = \infty$. It is somewhat easier to interpret γ than β, as the probability of an alien female being inseminated is simply γ times the probability of a conspecific female being inseminated. We shall call γ the "isolation ratio."

We shall now consider the implications of the hypotheses, discussed above, about the manner in which the mating preferences become effective. If males do not mate with alien females unless conspecific females are unavailable, we should expect to find no inseminated aliens until nearly all the conspecific females have been inseminated. Since this is not so, we must look for some further explanation. It might be that only a small proportion of the females present are receptive at any one time. This seems rather farfetched; it might, however, be tested by seeing what happens when only one or two males are present. On the other hand, the hypothesis might be altered to give a hypothesis which we will call H1 by supposing that the probability π_a of an alien female being inseminated is not zero but has some low value when conspecific females are available and has a higher value when only aliens are available. If we also suppose that the presence of conspecific females excites males and makes them more likely to mate, we will have a situation where π_a has one low value in the absence of conspecific females, another low value when conspecific females are available, and a higher value when conspecific females have been available but are so no longer. The balance between these opposing tendencies would then determine whether π_a would be increased or decreased when the number of conspecific females n_c was increased from zero to, e.g., ten. Introducing a second subscript to refer to the experiment number, the question is whether π_{a3}/π_{a2} is greater than or less than one. The data give[4] $p_{a3}/p_{a2} = 1.23$, but this does not differ significantly from one. This comparison was repeated, using aged flies to decrease the heterogeneity as explained above. When these vials were tested for heterogeneity they gave $\chi^2 = 19.56$ with 28 degrees of freedom, for $P = 0.85$, so that these results are much more reliable. This time $p_{a9}/p_{a8} = 1.36$ and this is significant, with $\chi^2 = 4.81$ and $P = 0.03$.

Before discussing H1 further we will consider an alternative hypothesis, H2. We now suppose that the isolation ratio $\gamma = \pi_a(n_c, n_a, t)/\pi_c(n_c, n_a, t)$ is a constant independent of n_c, n_a, and t. Evidently this cannot hold for all possible n_c, n_a and t; for example, if t is very large and n_c and n_a small, then π_c, π_a and hence γ will tend to 1; however γ may be essentially constant over a wide range of values. We shall not specify any definite forms for the functions π_c and π_a but merely suppose that in the xth experiment π_c has a fixed value π_{cx} and π_a has a fixed value π_{ax}. We temporarily ignore the heterogeneity of the data. Hypothesis H2 now reduces to H2′, where we assume that π_a/π_c has a common value γ_x for each vial of experiment x, and the hypothesis states that all the γ_x are equal. We represent the common value of the γ_x by γ.

Evidently γ_x involves a comparison between two kinds of females and hence cannot be computed for experiments 1 and 2, and therefore they cannot be used in testing H2′. For the other experiments, under the assumption only (i.e., when the γ_x need not be equal), the best estimates for π_{cx}, etc., are p_{cx}, etc., where p_{cx} is the proportion of conspecific females fertilized in all vials of the xth experiment; and the best estimate of γ_x is $g_x = p_{ax}/p_{cx}$. The g_x are given in table 1.

When H2′ is true, the p's are no longer the best estimates. We may now obtain π_{ax} from the relation that under H2′, $\pi_{ax} = \gamma\pi_{cx}$ but we still require joint estimates of $\gamma, \pi_{c3}, \pi_{c4}, \ldots, \pi_{c7}$. The method of maximum likelihood[5] leads to the set of six non-linear equations

$$\left.\begin{aligned}
&\sum_{x=3}^{7}\left[\frac{F_{ax}}{\gamma} - \frac{\pi_{cx}(N_{ax} - F_{ax})}{1 - \gamma\pi_{cx}}\right] = 0 \\
&\left[\frac{F_{cx} + F_{ax}}{\pi_{cx}} - \frac{N_{cx} - F_{cx}}{1 - \pi_{cx}} - \frac{\gamma(N_{ax} - F_{ax})}{1 - \gamma\pi_{cx}}\right] = 0 \quad (x = 3, 4, \ldots, 7).
\end{aligned}\right\} \quad (1)$$

It is not practicable to solve these equations exactly, but a method of successive approximations gives the estimate $g = 0.1958$ and estimates of the π_{cx} which lead to the "expected values" that are given in table 1. The ordinary χ^2 test is not strictly applicable here because the constraints are non-linear. However the likelihood ratio statistic,[6] λ, is easily calculated and, for samples as large as this, $-2 \log_e \lambda$ should be distributed approximately as χ^2 with 4 degrees of freedom.[7]

To compute $-2 \log_e \lambda$ we need the expression

$$(-2 \log_e 10) \sum_{x=3}^{7} [(F_{cx} + F_{ax}) \log \pi_{cx} + F_{ax} \log \gamma_x + (N_{cx} - F_{cx}) \log (1 - \pi_{cx}) + (N_{ax} - F_{ax}) \log (1 - \gamma_x\pi_{cx})]. \quad (2)$$

(All logarithms are to the base 10 unless otherwise indicated.) Now let L_1 be the value of (2) when we replace γ_x by γ and then replace γ, π_{c3}, $\pi_{c4}, \ldots, \pi_{c7}$ by the numerical estimates obtained fom equations (1). Also, let L_2 be the value of (2) when we replace $\gamma_3, \ldots, \gamma_7, \pi_{c3}, \ldots, \pi_{c7}$, by the sample values given in table 1. Then $-2 \log_e \lambda = L_1 - L_2$ We obtain the result $-2 \log_e \lambda = 9.0$, corresponding to a probability of about 0.061.

Discussion.—In nature, H2 would mean essentially that there is one probability of copulation when a male meets one of his own females and another (smaller) probability when he meets an alien female, while under H1 we have the added complication that his willingness to mate with the alien is reduced by the presence of receptive conspecific females. It should be noted that we have spoken throughout as if it were the male who did the choosing. This has been done solely for economy of words. Actually it is not known whether it is the males or the females or both which exercise the discrimination. It may be that the alien females reject the males, and that H1 means that the males are more likely to persevere if no conspecific females are present. The end result will be the same in any case. The important thing is that, under H2, π_a and π_c both decrease at the same rate, as n_c increases, while, under H1, π_a decreases more rapidly than π_c so that γ decreases. The data also require the assumption that males become excited if conspecific females are present, and then are more likely to mate with alien females. This additional effect could be superimposed on either H1 or H2, but would have to be more extreme to override the effect of H1. Since there is no other evidence in favor of H1, it seems that the simpler hypothesis H2 should be accepted. We have seen that, ignoring heterogeneity, the probability of the data arising by chance under H2′ is about 0.06. The heterogeneity would tend to increase the probability of more extreme values of the statistic $-2 \log_e \lambda$, and hence to further reduce the need of rejecting H2′. On the other hand, the heterogeneity would also tend to overshadow any real differences in the γ_x and hence make the test less sensitive. Table 1 shows on its face that the g_x do vary over a range of 2 to 1, but in an irregular manner rather than in the regular way which we would expect under H1. The actual variation in the γ_x could be even greater than this, but it is not likely that very large systematic changes occur. All things considered it seems best to accept the simpler hypothesis H2, since it is not contradicted by the data, and indeed seems to fit them better than H1. In any event the simple hypothesis that males only mate with alien females when their own females are unavailable seems untenable, and its modification, H1, is simply a more complicated version of H2.

Results with Two Strains of D. prosaltans.—Experiments were also carried out with the same number of flies as in experiments 1 to 7, but using flies of the Chilpancingo (Mexico) strain of *D. prosaltans* instead of *D. persimilis* and flies of the Bertioga (Brazil) strain of *D. prosaltans* instead of *D. pseudoobscura.* As shown by Dobzhansky and Streisinger,[8] males of both these strains exhibit a strong preference for mating with Chilpancingo females. Freshly hatched flies were kept together in an incubator at 25$^1/_2$°C. for approximately 3 days, whereupon all the females were dissected and their seminal receptacles examined for sperm. These experiments were performed in August–September, 1944. The results are summarized as experiments 1′ to 7′ in table 2.

TABLE 2

D. prosaltans. NUMBERS OF INSEMINATED FEMALES OBSERVED WHEN 10 CHILPANCINGO MALES WERE PLACED WITH n_c CHILPANCINGO FEMALES AND n_a BERTIOGA FEMALES

EXPT. NUMBER	NO. OF CONSPECIFIC PER VIAL	NO. OF ALIEN PER VIAL	CHILPANCINGO			BERTIOGA			ISOLATION INDEX	ISOLATION RATIO
			FERTILIZED	UNFERTILIZED	TOTAL	FERTILIZED	UNFERTILIZED	TOTAL		
x	n_{cx}	n_{ax}	F_{cx}	$N_{cx}-F_{cx}$	N_{cx}	F_{ax}	$N_{ax}-F_{ax}$	N_{ax}	b_x	g_x
1′	10	0	57* 63.33*	33 36.67	90					
2′	0	10				14 14.00	86 86.00	100		
3′	10	10	72 72.73	27 27.27	99	2 2.06	95 97.94	97	0.945	0.0284
4′	20	10	116 66.67	58 33.33	174	1 1.15	86 98.85	87	0.966	0.0172
5′	40	10	156 39.39	240 60.61	396	1 1.02	97 98.98	98	0.950	0.0259
6′	10	20	57 72.15	22 27.85	79	3 1.94	152 98.06	155	0.948	0.0268
7′	10	40	64 66.67	32 33.33	96	12 3.05	381 96.95	393	0.912	0.0458

* For each experiment, the top line gives the observed number of flies and the bottom line gives the observed percentages.

In these experiments the observed frequencies of heterogamic fertilization are so low that their sampling fluctuations would overshadow any reasonable changes in the γ_x. All that can be said is that the data in experiments 3′ through 7′ do not contradict H2′. For such low γ values large numbers of vials would have to be used to obtain good results. The only significant result is the considerable increase of heterogamic matings when no conspecific females are present. We have $p_{a3}'/p_{a2}' = 0.147$, with $\chi^2 = 7.43$, using Yates' correction for continuity, and $P = 0.006$. This is probably a real effect, although it might be due to the heterogeneity between vials. If real, this effect is more consistent with H1 than with H2. It could be at least partly explained under H2 as follows. Suppose π_c and π_a depend on an "effective population density" with an added female affecting π_a in proportion to her chance of insemination; i.e., if, as suggested above, we consider π_a to depend on a linear combination, $n' = dn_c + en_a = d[n_c + (e/d)n_a]$, of n_c and n_a, then e/d will be equal to γ. Now if $\pi_a(n', t)$ decreases rapidly as n' increases from 0 to 2 or 4, and thereafter decreases less rapidly, then if γ is small, as it is here, 10 γ will be small, and increasing n_c from 0 to 10 will reduce π_a considerably. There is as yet no experimental evidence on the behavior of π_c and π_a for small n_c by which this conjecture could be tested.

Thus the difference in behavior in the two series of experiments need not necessarily imply different causal systems. However, it would not be surprising if they were different, since in one case we are dealing with sexual isolation between two species and in the other with a one-way sexual preference between two strains of a single, different species.

Summary.—Results obtained by placing *D. persimilis* males with varying proportions of *D. persimilis* and *D. pseudoobscura* females admit of the hypothesis that the ratio of the probability of a heterogamic mating to the probability of a homogamic mating is a fixed constant independent of these proportions. However, the possibility of some decrease in this ratio when many *D. persimilis* females are present cannot be rejected. Because of small numbers of heterogamic matings, similar experiments with two strains of *D. prosaltans* furnish little evidence on this point. For *D. persimilis-D. pseudoobscura*, heterogamic matings are significantly more frequent in vials containing 10 females of each of the two species than in vials containing 10 alien females only, while for *D. prosaltans* they are significantly less frequent.

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[*] Experimental data by Th. Dobzhansky, mathematical analysis by H. Levene.

[1] Mayr, E., and Dobzhansky, Th., these PROCEEDINGS, **31**, 75–82 (1945).

[2] For simplicity, we shall use the terms "conspecific" and "alien" regardless of whether the flies differ as to species (*D. persimilis* and *D. pseudoobscura*) or merely as to strain (geographic strains of *D. prosaltans*).

[3] Stalker, H. D., *Genetics*, **27**, 238–257 (1942).

[4] Throughout this paper Greek letters are used for population values (i.e., values characteristic of a given experimental set-up) and the corresponding Roman letters are used for estimates based on the sample of flies observed. Thus the best estimate of π_{c3} is $p_{c3} = F_{c3}/N_{c3}$.

[5] Fisher, R. A., *Statistical Methods for Research Workers*, London, Sec. 53 ff., 2nd (1928) and later editions.

[6] Neyman, J., and Pearson, E. S., *Biometrika*, **20A**, 175–240 and 263–294 (1928).

[7] Wald, A., *Trans. Am. Math. Soc.*, **54**, 428–482 (1943).

[8] Dobzhansky, Th., and Streisinger, G., these PROCEEDINGS, **30**, 340–345 (1944).

THE THREE-DIMENSIONAL SHAPES OF BUBBLES IN FOAMS

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The three-dimensional shapes of bubbles in foams have been discussed by scientists in diverse fields for nearly three centuries, but apparently exact studies have not heretofore been made.

Even in his *Micrographia*, published in 1665, Robert Hooke,[1] the first