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ABSTRACT

Certain aspects of mating behavior in two different types of Drosophila melanogaster females (Basc and Bv) and wildtype males (Samarkand) have been investigated. In one series of experiments (Series A), individual males were permitted to choose between two females of the two different genotypes only. In the second series (Series B) started about three years after the termination of the first, individual males were permitted to choose between (a) two females from the Bv strain, (b) two females from the Basc strain, or (c) a Basc and a Bv female. The three flies were observed over a period of 2-1/4 hours from the time they were placed together in a vial. No female copulated twice during this period.

When the males copulated for the first time, the choice of partners was random; approximately 50% of the first copulations were with Bv and 50 % with Basc females. When the first copulations were with Bv females, the type of the second female in the vial had little influence on the frequencies of the second copulations. However, when the Basc females were the first partners, the type of the second female in the vial did influence the frequencies of the second copulations; the males copulated more frequently a second time when the second was a Bv rather than a Basc female. Factors that might have accounted for the differences are discussed, including olfaction.

When all four mating combination Series B (Basc-Basc, Basc-Bv, Bv-Bv, Bv-Basc) were compared, the premating periods hardly differed from each other, nor did the intermating periods. Both of these periods were shorter than the respective periods in Series A. Genetic changes (mutation and recombination) occurring during the three year interval may have caused the females to be more receptive to the males in the later than

in the earlier experiments. In Series A, the premating and intermating periods were both shorter when the first copulations were with Basc rather than with Bv females. Changes in heterogeneity may have decreased the advantage of the Basc females in these two categories.

The durations of the second copulations were longer than those of the first with both types of females, and there was a positive correlation between the durations of the first and second copulations. The durations of the copulations depended to a great extent on the males but also were influenced by the females, as were the premating and intermating periods.

Nearly twice as many offspring resulted from the first as from the second copulations.

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I. INTRODUCTION

Recent experiments by Hildreth have shown that when individual wildtype Drosophila melanogaster males were placed together with two females, either both of the same genotype (both having white eyes or both having red eyes) or each of a different genotype (one having red and the other having white eyes), the males copulated much more frequently with the white-eyed females than with the red-eyed females. Both the premating and the intermating periods before copulations with white-eyed females were shorter than before copulations with red-eyed females. With each type of female, the average durations of the second copulations by the males were longer than the first, and the durations of the second copulations were positively correlated with the durations of the first. The investigations reported here are concerned with similar aspects of mating behavior when males of the same type but females of different types from those used in the above experiments were studied.

Dobzhansky and Koller (1938) carried out experiments on sexual isolation in Drosophila. Since then there have been several reports concerned with various aspects of courtship and mating behavior and with the evolutionary significance of these behavioral patterns in the genus Drosophila.

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Extensive reviews of the literature have been made by Petit in 1958, Hoenigsberg and Santibanez in 1959 and 1960, Montalenti in 1959 and Hildreth in 1961.

II. STOCKS

Three different stocks of Drosophila melanogaster were used. Two provided the females, and the third the males. Each female presented a specific phenotype, which was readily discernible to the unaided eye.

Basc females (also called M-5 and Muller-5 in the literature) have the grayish wildtype body color but bar-shaped (B) and apricot-colored eyes (w^a) instead of the round, red eyes of the wildtype. These females also carried an inversion (sc^{S1} InS sc^8) and genetically were homozygous for the inversion-carrying X chromosome "sc^{S1} B InS w^a sc^8".*

Bv females have yellow bodies (y^2/y) and kidney-shaped (B/+) vermilion-colored ($v\ w^a/v\ +$) eyes. One X chromosome carried an inversion (sc^{S1} In49 sc^8), and the other X chromosome had the long arm of the Y chromosome attached to it (. Y^L); this X chromosome also carried the mutant for forked bristles (f). Genetically these females were "y^2 sc^{S1} B $+^f$ In49 v w^a sc^8/y $+^{w^a}$ v f $+^B$. Y^L".*

Samarkand wildtype males have the wildtype grayish body color and the wildtype round red eyes. The Samarkand stock had been kept in various laboratories for 239 generations with brother and sister matings and then had been kept in our laboratory in mass culture throughout these experiments.

The experiments were done in two series and about three years had elapsed between the two. The stocks used in the second series were derived from those used in the first. Undoubtedly genetic changes without recognizable phenotypic expression had occurred during this time. These changes may have been responsible for certain differences between the results of the two series. In the Bv stock, a lack of males in one generation made it necessary to outcross the Bv females to males carrying genetically marked crossover-suppressor chromosomes to preserve the X chromosome and autosomes from the original stock.

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*Stocks of the above types were kindly supplied to us by Dr. H. J. Muller, Indiana University, Bloomington, Indiana.

III. SERIES A. PRESENTATION OF SINGLE MALES WITH TWO FEMALES OF DIFFERENT STRAINS

A. Procedure

Individual males were permitted to choose between a Basc and a Bv female. All flies were approximately 24 to 32 hours old. The technique of observation was the same as that reported earlier by Hildreth except that the flies were observed over a period of 2-1/4 hours in each experiment instead of 2 hours. The types of mating, the premating and intermating periods, and the durations of the copulations were recorded.

When a male had completed copulating with both females, the flies were etherized, each female was placed in a separate vial and the male was discarded. When the females had awakened, each was shaken into a half-pint culture bottle which was then labeled as to the type of female, the number of the male, and the time that the female was placed in the culture bottle. Forty-eight hours later, the females were removed and discarded. Twelve days after this, the culture bottles were checked for fertility and the numbers of offspring in the fertile cultures. Experiments were carried out always in the same place in the early afternoon. The experimental area received light from both standard incandescent lamps and from the windows facing north. Throughout the series of experiments, the room temperature varied from 21.6 to 27.0°C, but in individual experiments the range was much less. At all other times while being stored or cultured, the flies were kept in incubators set at 26°C.

B. Results

Among 1177 vials each containing one Basc and one Bv female and one Samarkand wildtype male, about 27% (324) of the males copulated with both females, about 56% (662) copulated with one female each, and about 17% of the males did not copulate during the 2-1/4-hour period. No male copulated twice with the same female. The frequencies of both single and double copulations within a mating group varied from one experiment to another. Since the flies were relatively young when permitted to mate, a few hours difference in age might account for the observed variations. It is also possible that temperature and atmospheric conditions could influence these frequencies. In those trials in which the male copulated with both females

(see Table I) the chances of fertilization did not seem to be affected by the type of female. The two groups, with either the Basc or the Bv females mated first, hardly differed in the proportion of trials in which (a) both females were fertile, (b) only the first female was fertile, (c) only the second female was fertile, or (d) neither female was fertile. An homogeneity test of these data yielded a probability of 0.5 to 0.7 that chance fluctuations alone could have caused the differences in frequencies between the "Basc first" and "Bv first" groups.

Table I. Fertility when individual males copulated with both a Basc and a Bv female. One Basc and one Bv female were present in each vial with a Samarkand wildtype male.

Mating sequence	Double copulations	Double copulations checked	Fertility (%)			
			Both females	Only first of two females	Only second female	Neither female
Basc-Bv	213	173	82	14	3	1
Bv-Basc	111	97	85	11	2	2

Mating Preferences

First copulations: When the males copulated for the first time they showed no marked preference for either type of female. There were 491 cases in which the males copulated with Basc females only or with the Basc females first; 495 males copulated with Bv females only, or with Bv females first. The probability of such a difference occurring by chance alone is approximately 0.90. Santibanez and Waddington (1958) had found that yellow females were very receptive toward males of any kind. The yellow females used in our experiments were hardly more receptive to the wildtype males than were the nonyellow females.

Second copulation: There was a stronger tendency for the males to copulate a second time if the first copulation was with a Basc female, despite the fact that there was no preference for Basc over Bv as the first partner. Among the males that copulated with Basc females, 43.6% (213)

copulated afterwards with Bv females. Of the males that copulated with Bv females, only 22.6% (111) then copulated with Basc females. The probability that such a difference would occur by chance alone is much less than 0.0001 (χ^2=48.96, D.F. = 1).

Premating Periods

Since the males had little preference for one type of female over the other as the first partner, it was of interest to know whether the premating periods before the copulations with the Basc and Bv females were similar. Despite the lack of preference, the males did copulate sooner with the Basc than with the Bv females. When the first copulations were with Basc females, 40% (198/491) were begun within 15 minutes from the time the males were placed with the females. However, when the males copulated first with the Bv females, only 17% (82/495) of the copulations were initiated within the same period. The probability that such a difference would occur by chance alone is less than 0.0001 (χ^2=73.27, D.F.=1).

Intermating Periods

The intermating periods were shorter when the males copulated first with Basc females than when they copulated first with Bv females.

Within 15 minutes after the first copulations with Basc females, 41.3% (88/213) of the males had then begun copulations with Bv females. During the same interval, only 23.4% (26/111) of the males that copulated first with Bv females had then initiated copulations with Basc females. The probability that such a difference would occur by chance alone is less than 0.002 (χ^2= 10.24, D.F.=1).

Durations of Copulations

When individual males copulated twice, the estimates of the durations of the first copulations ranged from 5 to 20 minutes. Durations of the single copulations were within the same range. During second copulations with the Basc females, the range was increased to 25 minutes, and with one Bv female to 30 minutes. The average duration of the first copulations with Basc females was 13.6 minutes and with Bv females was 13.3 minutes (Table IIa). The average durations of the second copulations were significantly longer than the first being, 16.1 vs 13.6 minutes with the Basc females (Table IIc) and 16.5 vs 13.3 minutes with the Bv females (Table IId). It is seen that the type of female did not influence the durations of the first

copulations (Table IIa) or the second (Table IIb). It appears that the difference in durations between the first and second copulations depended on the male and not on the type of female.

The durations of the single copulations with the Basc females were longer than the durations of the singles with Bv females (Table IIa). The durations of the single copulations tended to be longer than the first of the double copulations. This was true in both the Basc and Bv series (Tables IIf and IIg).

There is a positive correlation between the first and second copulations in that an increase in the duration of the first copulation was followed by an increase in the second (Table III).

Numbers of Offspring

The two types of females used were of different genotypes and one would expect that, because of this, the numbers of offspring from the two types would not be the same. The Bv females on the average produced more offspring from the same mating sequence than did the Basc females. From the first copulations, 76 Bv females produced 7093 offspring, with an average of 93.3 per female, and 125 Basc females produced 10615 offspring, with an average of 84.9 per female ($P<0.0001$, $\chi^2 = 37.97$, D.F. = 1). The second copulations with Bv females produced 7221 offspring, with an average of 57.8 per female. The Basc females produced 7221 offspring, with an average of 45.7 per female ($P<0.0001$, $\chi^2 = 129.68$, D.F. = 1). The more important aspect of the offspring counts is the significant difference between the numbers of offspring from the first and second copulations by the males. In each case the numbers resulting from the first copulations were greater than those from the second copulations. The probabilities that chance deviations would account for the differences are much less than 0.0001 (D.F. = 1: Basc first vs Basc second, $\chi^2 = 1038.03$; Bv first vs Bv second, $\chi^2 = 839.43$).

Table II. Durations of copulations. One Basc and one Bv female were present with a Samarkand male in each vial.

Mating sequence	Duration (min) 5	10	15	20	25	30	Average duration (min)	Total vials	χ^2	D.F.	P
	Distribution (%)										
(a) Basc first	1	33	61	5	0	0	13.6	213	3.38	3	0.3-0.5
Bv first	3	31	62	4	0	0	13.3	111			
(b) Basc second	2	19	40	34	5	0	16.1	111	4.74	5	0.3-0.5
Bv second	2	10	52	29	6	1	16.5	213			
(c) Basc first	1	33	61	5	0	0	13.6	213	65.23	4	<0.0001
Basc second	2	19	40	34	5	0	16.1	111			
(d) Bv first	3	31	62	4	0	0	13.3	111	51.44	5	<0.0001
Bv second	2	10	52	29	6	1	16.5	213			
(e) Basc only	2	17	67	14	0	0	14.6	278	10.20	3	0.01-0.02
Bv only	0	25	62	13	0	0	14.1	384			
(f) Basc only	2	17	67	14	0	0	14.6	278	23.16	3	<0.0001
Basc first	1	33	61	5	0	0	13.6	213			
(g) Bv only	0	25	62	13	0	0	14.1	384	14.17	3	<0.003
Bv first	3	31	62	4	0	0	13.3	111			

Table III. Mean durations of copulations when individual males mated with both A Basc and a Bv female. One Basc and one Bv female were present in each vial with a Samarkand male.

Number of males	Duration (min) Basc first	Bv second	Male average	r*	b+	P
1	5.0	15.0	20.0			
70	10.0	15.9	25.9	0.119	0.174	0.05-0.1
131	15.0	16.6	31.6			
11	20.0	18.2	38.2			
	Bv first	Basc second				
3	5.0	13.3	18.3			
35	10.0	15.6	25.6	0.197	0.293	0.02-0.05
69	15.0	16.2	31.2			
4	20.0	21.3	41.3			
		Total		0.149	0.219	<0.01

*Here r is the correlation coefficient.

+Here b is the regression of second copulations dependent on the first.

IV. SERIES B. PRESENTATION OF SINGLE MALES WITH TWO FEMALES OF THE SAME STRAINS OR OF DIFFERENT STRAINS

A. Introduction

Many questions were left unanswered in the Series-A experiments. Interest focused on reasons why a male that first copulated with a Basc female was more likely to copulate again than a male that had copulated first with a Bv female. What would happen if, after the first copulation, a male was faced with another female of the same type as the first? To provide an answer to this and to other questions, individual males were allowed to choose between (a) two Basc females, (b) two Bv, females, or (c) between a Basc and a Bv female as in the original experimental Series A. As was stated earlier, there may have been changes in the stocks used, because of the three years which separated the experiments in Series A from Series B. However, the technique was the same in both series of experiments. The offspring which resulted from the copulations in the latter experiments were not counted.

B. Results

Frequency of Copulations

The percentages of males that (a) did not copulate, (b) copulated once, or (c) copulated twice are presented in Table IV. The differences between the frequencies of copulations with the three kinds of pairs of females are significant at the 0.5% level ($\chi^2 = 15.06$, D.F. = 4). The frequencies of copulations when two Bv females were present hardly differed from the frequencies when two Basc females were present ($P = 0.7$ to 0.8). The frequencies when a Basc and a Bv female were present were much greater than when two Basc ($P < 0.005$, $\chi^2 = 11.00$, D.F. = 2) or when two Bv females ($P = 0.01$ to 0.02, $\chi^2 = 8.54$, D.F. = 2) were present.

Mating Preference

First copulations: The incidence of first copulations was not affected by the type of female. When 305 males were tested with pairs of Basc females, 193 copulated at least once. Of the 267 males tested with Bv females 167 copulated at least once ($P = 0.8$ to 0.9). Among the 505 males tested with one Basc and one Bv female, 194 males copulated with Basc

Table IV. Percentage of copulations with two females and one Samarkand male present in each vial.

Females present	Distribution (%)			Total males available
	Neither female copulated	Only one female copulated	Both females copulated	
Basc-Basc	37	45	18	305
Bv - Bv	38	42	20	267
One Basc and one Bv	28	44	28	505

females as the first or only partner, and 167 with Bv females as the first or only partner (P = 0.1 to 0.2).

Second copulations: After the males had copulated once, the tendency to copulate again varied depending on the types of females in the vials (Table V). A comparison of the four possible mating sequences in regard to the frequency of single and double copulations yielded a significant deviation from homogeneity (P = 0.02, $\chi^2 = 9.79$, D. F. = 3).

Table V. Percentage of individual males that copulated twice.

Females present	Number of males that copulated	Number of males that copulated twice	Percent of males that copulated twice
Basc-Basc	193	56	29
Bv-Bv	167	54	32
One Basc and one Bv:	194 Basc first	86	44
	167 Bv first	55	33

When males were tested with Basc-Bv pairs, the frequency of second copulations was much higher when the first were with Basc and the second with Bv than when the first were with Bv and the second with Basc females (Table V). The differences are significant at about the 3% level ($\chi^2 = 4.97$, D. F. = 1).

After the males had copulated with Bv females, the type of the second female present had little influence on the frequency of the second copulations (Table V). The probability that the differences in frequency between the Bv-Basc and the Bv-Bv sequences would occur by chance deviations alone is 0.90 to 0.95. Contrary to this, when the first copulations were with Basc females, the type of the second female present influenced greatly the frequency of the second copulations (Table V). The probability that chance deviations alone would have accounted for the difference in frequency between the Basc-Basc and the Basc-Bv sequences is less than 0.002 (χ^2=9.88, D.F.=1).

Premating Periods

As in Series A, the analysis of the data was based on the proportion of males that had begun their first copulations within 15 minutes after they had been placed with the females. The durations of the premating periods appeared to be independent of the types of females involved, as the differences between the durations (Table VI) were slight and in all cases could have resulted from chance deviations.

Table VI. Premating periods. Percentage of males that had begun their copulations within 15 minutes after being placed with the two females.

Female mated first	Females present	Percent Within 15 min	Percent After 15 min	Total males
Basc	Basc-Bv	64	36	194
Basc	Basc-Basc	57	43	193
Bv	Bv-Basc	55	45	167
Bv	Bv-Bv	61	39	167

	χ^2	D.F.	P
Bv-Basc vs Basc-Bv	3.36	1	0.05-0.1
Bv-Bv vs Basc-Basc	0.60	1	0.3-0.5
Basc-Basc vs Basc-Bv	2.31	1	0.1-0.2
Bv-Bv vs Bv-Basc	1.23	1	0.2-0.3

Intermating Periods

There were no significant differences between the intermating periods of the various groups (Table VII). This indicates that the type of female, whether the first or the second partner of the male, did not greatly influence these periods.

Table VII. Intermating periods. Percentage of males that began their second copulations within 15 minutes after termination of the first.

First partner	Females present	Percent: Within 15 min	Percent: After 15 min	Total males
Basc	Basc-Basc	52	48	56
Basc	Basc-Bv	50	50	86
Bv	Bv-Basc	53	47	55
Bv	Bv-Bv	39	61	54

	χ^2	D.F.	P
Bv-Basc vs Basc-Bv	0.10	1	0.7-0.8
Bv-Bv vs Basc-Basc	1.80	1	0.1-0.2
Basc-Basc vs Basc-Bv	0.04	1	0.8-0.9
Bv-Bv vs Bv-Basc	2.13	1	0.1-0.2

Durations of Copulations

The durations of the copulations within the various groups ranged from five to 25 minutes for the first or only copulations and from five to 35 minutes for the second copulations. For the statistical comparisons, individuals were grouped according to the durations of copulations, as shown in Table VIII.

Table VIII. Durations of copulations. Two females and one wildtype male were present in each vial.

Females present	First partner	Total males	Duration (min) 5	10	15	20	25	Average	Second partner	Duration (min) 5	10	15	20	25	30	35	Average
			Distribution(%)							Distribution (%)							
Basc-Bv	Basc	86	2	11	71	16	0	15.1	Bv	8	18	45	21	7	1	0	15.2
	Bv	55	2	22	62	14	0	14.5	Basc	16	11	33	35	3	2	0	15.2
Basc-Basc	Basc	52	4	8	81	7	0	14.6	Basc	8	19	19	37	15	2	0	16.9
Bv-Bv	Bv	51	2	20	69	6	3	14.5	Bv	16	10	41	25	4	2	2	15.3
		Single copulations															
Basc-Bv	Basc	108	1	8	70	19	2	15.6									
	Bv	112	0	19	70	11	0	14.6									
Basc-Basc	Basc	137	1	17	57	23	2	15.4									
Bv-Bv	Bv	113	1	20	59	19	1	15.0									

Single copulations: There were no significant differences between the durations of the single copulations with Basc and Bv females when males could choose between the two types ($P = 0.05$ to 0.1). There was little influence of the second type of female present on the durations of the single copulations (Basc-Bv vs Basc-Basc, $P = 0.1$ to 0.2; Bv-Basc vs Bv-Bv, $P = 0.2$ to 0.3).

First of double copulations: There were no significant differences between the durations of the first of the double copulations regardless of whether the Bv or Basc females copulated first ($P > 0.07$ in each case). The type of the second female in the vial had little influence on the durations of the first copulations. The probability for the differences between the Basc-Bv and the Basc-Basc combinations occurring by chance alone is 0.3 to 0.5 and between the Bv-Basc and Bv-Bv combinations is 0.7 to 0.8.

Second of double copulations: The durations of the second copulations with the Basc females were influenced very little by the type of the first female with which the males had copulated (Bv-Basc vs Basc-Basc, $P = 0.1$ -0.2). The type of the first female with which the male copulated had little influence on the durations of the second copulations with the Bv females (Basc-Bv vs Bv-Bv, $P=0.3$-0.5). In two of the analyses comparing the second copulations of the Basc with the Bv females, there were no significant differences in the durations (Bv-Basc vs Basc-Bv, $P = 0.1$-0.2; Bv-Basc vs Bv-Bv, $P = 0.8$-0.9).

In two of the analyses there were significant differences in the durations of the second copulations, and each of these involved the Basc-Basc combination. When the first copulations were with the Basc females, the second copulations with Basc females tended to be longer than the second with the Bv females ($P<0.05$, D.F. = 5, $\chi^2 = 11.63$; χ^2 of 11.07 is at 5% level of significance). When the first and second copulations were with the Basc females, the second copulations tended to be longer than when the first and second copulations were with the Bv females ($P<0.05$, D.F. = 6, $\chi^2 = 12.63$; χ^2 of 12.59 is at the 5% level of significance). Possibly the significance of these two latter cases is not of great importance, because 11 comparisons have been made on the data in this section, and nine of the comparisons yielded probabilities well above the 5% level of significance. Only these last two yielded probabilities just below the 5% level.

First vs second copulations: The comparisons of the first and second copulations with Basc females showed that the second copulations were of greater duration than the first. In each case the probability for the difference occurring by chance was less than 0.0001 (Basc first-Bv vs Bv-Basc second, Basc first-Bv vs Basc-Basc second, Basc first-Basc vs Basc-Basc second, Basc first-Basc vs Bv-Basc second).

The results of the comparisons between the durations of the first and the second copulations with the Bv females are not as consistent as those with the Basc females. The second copulations with Bv females (Basc-Bv) were not significantly different from the first with Bv females (Bv-Basc). The probability for chance deviations accounting for the difference is approximately 0.08. In the other comparisons, the durations of the second copulations were significantly longer than were the first ones (Bv first-Basc vs Bv-Bv second, $P = 0.02$; Bv-Bv first vs second, $P < 0.005$; Bv first-Bv vs Basc-Bv second, $P = 0.02$). In Series A it was observed that as the average durations of the first copulations increased there was an increase in the duration of the second copulation (Table III). Here also, there was a positive correlation between the durations of the first and second copulations in each case (Table IX) although not as pronounced as in Series A.

Single vs first of double copulations: When the males were present with two Basc females, the first copulations were significantly shorter in duration than were the single copulations ($P < 0.01$, $\chi^2 = 13.35$, D.F. = 4; χ^2 of 13.277 for 1% level of significance). In the other comparisons there were no significant differences between the durations of the first and the single copulations. The probability that chance deviations alone could have accounted for the differences was greater than 10% in each case, and in one case was between 50% and 70%.

Table IX. Average durations of copulations when individual males copulated with both females.

Number of males	Duration (min)			r*	b+	P
	Basc first	Bv Second	Male Average			
2	5.0	10.0	15.0	0.277	0.483	0.01-0.02
9	10.0	12.8	22.8			
61	15.0	15.2	30.0			
14	20.0	17.5	37.5			
	Basc first	Basc second				
2	5.0	12.5	17.5	0.073	0.163	0.6-0.7
4	10.0	18.8	28.8			
42	15.0	16.9	31.9			
4	20.0	17.5	37.5			
	Bv first	Basc second				
1	5.0	15.0	20.0	0.099	0.181	0.4-0.5
12	10.0	15.0	25.0			
34	15.0	14.7	29.7			
8	20.0	17.5	37.5			
	Bv first	Bv second				
1	5.0	20.0	25.0	0.139	0.256	0.3-0.4
10	10.0	15.0	25.0			
35	15.0	14.4	29.4			
3	20.0	21.7	41.7			
2	25.0	20.0	45.0			
		Total		0.154	0.291	0.01-0.02

*Here r is the correlation coefficient.

+Here b is the regression of second copulations dependent on the first.

V. DISCUSSION

Series A vs Series B

As was stated earlier, approximately three years had elapsed between the times that the first and second series of experiments were carried out. If, as Bösiger pointed out in 1960, the selective advantage or disadvantage in sexual competition is caused not by the mutant marker genes per se but by the vigor of the males and females involved and if, in turn, the vigor is influenced by the degree of heterogeneity, then over this period of three years one might expect that there would be differences between the premating periods, intermating periods, durations of copulations, and mating frequencies of the two series of experiments. Even though the inversions in the X chromosomes and the mutant genes causing visible effects remained the same over this period, mutations causing nonvisible effects could have arisen in the X chromosomes and autosomes. In addition, recombination of genetic material may have occurred. Thus, even though they appeared phenotypically identical, the stocks in the experiments in Series B could have been much different from the stocks of the Series-A experiments.

The premating periods before copulations with both the Basc and the Bv females were shorter in the second than in the first series when individual males were permitted to choose between a Basc and a Bv female for the first partner. In Series A, 40% of the copulations with Basc females were begun within 15 minutes after the flies were placed together; in Series B, 64% of the copulations with Basc females were initiated within the same interval. The probability that chance deviations alone would have accounted for this difference is less than 0.0001 (χ^2=32.58, D.F.=1). When the males copulated first with Bv females, only 17% of the copulations in the earlier series were initiated within the first 15 minutes. However, in the second series, 55% were begun during the same interval. As in the previous case, the probability that chance deviations alone would account for the differences is much less than 0.0001 (χ^2 = 96.11, D.F.=1).

The intermating periods in the experiments of Series A and B differed only slightly when the first copulations were with Basc and the second were with Bv females. In Series A, 41% of the second copulations were initiated within 15 minutes after the termination of the first copulations. In Series B, 50% were begun during this interval (P = 0.1-0.2). However, when

the first copulations were with Bv and the second with Basc females, the frequency of males initiating second copulations within 15 minutes after termination of the first was increased from 21% in Series A to 53% in Series B. The probability that chance deviations alone would have accounted for this difference is less than 0.0002 ($\chi^2 = 14.32$, D.F. = 1).

The durations of copulations were so similar in two cases that differences between the durations in Series A and B could have been caused by chance fluctuations (Bv first, P = 0.05-0.1; Bv only, P = 0.3-0.5). In the other four comparisons, chance fluctuations alone would not have accounted for the differences. When the males copulated with Basc females first or only with Basc females, the durations in Series B were greater than in Series A (Basc first, $P < 0.01$; Basc only, P = 0.01-0.02). The durations of the second copulations, whether with Basc or Bv, were shorter in Series B than in Series A (Basc second, $P < 0.01$; Bv second, P = 0.02-0.05).

It would appear from the above tests that during the three-year interval between the two series of experiments, genetic changes had occurred and heterogeneity had been increased. Thus a generally increased sexual vigor of the flies may have accounted for the shortened premating and intermating periods in Series B. Environmental changes such as temperature and climatic conditions or even possible slight differences in culture conditions cannot be ruled out entirely as causes for the observed differences between the experiments of Series A and B. However, it seems unlikely that these factors were the causes. An increased vigor also may have been responsible for the increases (though not significant in two cases) in the durations of the first and of the single copulations. The fact that the durations of the second copulations decreased in both instances is not incompatible with the viewpoint that the durations of the first and single copulations increased because of an increased vigor, if one assumes that the changes came about in the females and not in the wildtype males. An increased vigor of the females might permit long copulations, if the first copulations for the males, but unless the vigor of the males had also increased the durations of the second copulations would be shorter in the experiments in Series B than in Series A. If this were true, one might expect little change in the average total durations of copulations by the males in Series A and B. When the males copulate with Basc females first and Bv second, the total average durations for the males were 30.1 (Series A)

and 30.3 minutes (Series B). When the first copulations were with Bv females and the second with Basc, the total average durations for the males were 29.4 (Series A) and 29.7 minutes (Series B). The increased vigor of the females and lack of increase in vigor of the males would account for the stronger positive correlations between the durations of the first and second copulations in the earlier series than in the later series of experiments.

The frequency of copulations by the males was significantly greater in the earlier experiments (56%) than in the later ones (50%). Chance fluctuations alone would not have been responsible for the difference ($P = 0.002$, $\chi^2 = 9.85$, D.F. = 1). This does not fit very well with the idea of increased vigor or increased receptivity of the female, as one might expect the frequency of copulations to increase simultaneously with an increase in vigor.

In Series A approximately 50% of the males copulated with Basc females as the first or only partners and in Series B 54% copulated first or only with Basc females. The difference is not significant ($P=0.2-0.3$) and in each case the choice between Basc and Bv females was random. After the males had copulated with Basc females, the frequencies of copulations with Bv females differed only slightly: 43% in Series A and 44% in Series B copulated a second time ($P=0.8-0.9$). When the first copulation were with Bv females, however, the frequency of subsequent male copulation with Basc females was higher in the later (33%) than in the earlier series (22%) of experiments. The probability that chance fluctuations would have caused the difference is less than 0.01. It would seem that the sexual vigor or receptivity of the Basc females had increased more than that of the Bv females, but not enough to change greatly the random selection of a partner when both types of females were present.

Series B

Mating preference: In choosing the first partner, the males copulated nearly as frequently with one type of female as with the other, indicating random selection. However, after the male had copulated once, the choice of a second partner could no longer be at random; the first female with which he had copulated would not copulate again within the time limit of the experiment so that the only choice was whether or not to copulate with the second female. Thus the question arose as to the frequency of second copulations and as to the causes of the observed differences in frequency.

The males copulated more frequently a second time when the first partner was a Basc and second a Bv female than in the reciprocal crosses (in Series A as well as B). When all four mating combinations were compared as to the frequency of second copulations (Table V), the differences were significant at approximately the 2% level, the significance being caused mainly by the differences between the Basc-Basc and Basc-Bv sets. A separate analysis of these two sets showed that the second copulations were significantly more frequent when the second female in the vial was of the Bv rather than the Basc genotype ($P < 0.003$, $\chi^2 = 9.88$, D.F. = 1). Contrary to this, when the first copulations were with Bv females the frequencies of second copulations, whether with Basc or Bv females, were not significantly different ($P = 0.9$-0.95).

On the basis of the cases in which both types of females were present, one might wonder whether the copulations with the Basc females were not possibly as successful in releasing physiological tension as were those with the Bv females. This would result in an increased frequency of second copulations after once having copulated with Basc females. Conversely, one could assume that the copulation with the Basc female might be more stimulating or less exhausting in some way than one with a Bv female. Thus, the threshold of the mating drive in the male might be lowered so that he would tend to copulate more frequently a second time after having copulated with a Basc rather than with a Bv female. From these hypotheses one would expect that after the males had copulated with Basc females, the second copulations would occur in nearly equal frequencies, regardless of the type of the second female present. However, the analysis of the Basc-Basc versus the Basc-Bv data indicates that these assumptions are not valid, because the second copulations were much more frequent when the second females were Bv rather than Basc.

Sturtevant (1915) observed that pairs of flies would mate sooner if placed in vials in which copulations had just occurred than would pairs placed in clean vials, leading to the conclusion that olfaction affects the mating drive. Mayr (1950) removed the antennae from female Drosophila, placed them with males of one or more different types and observed the mating behavior of these and control flies. The results indicated that a chemical was released by the male which affected the mating threshold of the females. Such being the case, in our experiments one could assume that prior to the first copulation the females were equally sensitive to the scent from the male.

However, after a copulation the second females reacted differently to the scent and the reaction depended both on the first female that copulated and on the second female present. This assumption seems warranted because the males copulated with 32% of the Bv females secondly when the first copulations were with Bv females and 33% of the Basc females secondly when the first copulations were with Bv females. However when the first copulations were with Basc females, the frequency of second copulations was influenced greatly by the type of the second female present. Bösiger in 1960 indicates that Mayr's results might have been caused by the shock of the operation in removing the antennae rather than loss of olfactory sense per se. Petit in 1959 minimizes the role of olfaction as an aid for Drosophila in selecting a partner and feels that wing-vibration patterns are more important. The present works as well as earlier work by Hildreth indicates that olfaction may be important in the mating drive; the role of wing vibration patterns was not studied so that the importance of these cannot be evaluated in relation to the present work.

Premating periods: As shown in Table VI, the premating periods of the four mating combinations were similar to each other. This correlates well with the observation that one type of female was chosen nearly equally as frequently as the other for the first mating partner. In Series A the premating periods before copulations with Basc females were significantly shorter than before copulations with Bv females, even though there was little difference between the frequencies of Bv and Basc females as first mating partners. General reasons for the changes in premating periods were discussed above. In earlier experiments, Hildreth had observed that when wildtype males from the same stock as used here were given the choice between red-eyed and white-eyed females, the white-eyed females were the first partners four times more frequently than were the red-eyed females. The premating periods before copulations with white-eyed females were also significantly shorter than before copulations with red-eyed females, indicating that the durations of premating periods are correlated with the receptivity of the females.

Intermating periods: The four mating combinations had intermating periods similar to each other (Table VII), regardless of which type of female had copulated first or which second. This was true despite the fact that after the males had copulated with Basc females, the second copulations were more

frequent with Bv than with Basc females. One might expect from the results in Series A that, because the intermating periods were shorter in the Basc-Bv than in the Bv-Basc mating combinations, it would also follow that the second copulations would be more frequent in the Basc-Bv combination, as was found. However in Series B, even though the second copulations were more frequent with Bv than with Basc females, when the first had been with Basc females, the similar intermating periods suggest that whether or not a second copulation would occur was based on something other than a time factor. There seemed to be a definite preference influenced by the type of the second female in the vial.

Duration of copulations: In general, the durations of the single copulations with Basc and Bv females were similar, and the durations of the first copulations were similar to each other. In one instance (Basc-Basc) the duration of the single copulations was significantly longer than the first of the double copulations, but in all other analyses there were no significant differences. The second copulations with Basc and Bv females were also similar to each other in duration (there were two instances in which significance was doubtful, with $P = 0.05$ in each). The second copulations were longer than were the first. Apparently the durations depended on the male more than on the female, according to the evidence in Series B. However, in Series A the single copulations with Basc females were longer than were those with Bv females. It was shown that the durations of the copulations in Series A and B were significantly different from each other. These differences were attributed to changes in the genetic background of the females and therefore the durations of the copulations must also depend on the females. Here as in Series A, the durations of the second copulations were positively correlated with the durations of the first. Also, in earlier work Hildreth showed that the durations of the second copulations were positively correlated with the durations of the first.

The present work makes it seem advisable that one be extremely careful in comparing his own results from experiments on sexual preference (in Drosophila at least) with results from other laboratories, even though the same genetic markers be used, because various degrees of heterogeneity between the stocks, and not the markers causing the visible effects, may account for the differences observed.

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